Vol. 106
No. 2756

Pages 379-404

October 24, 1947

Science

THE SCIENTISTS NEWSWEEKLY

Kon-Tiki returning to San Francisco with her crew after having traveled the 4,300 odd miles from Callao, Peru, to Raroia in the Tuamoto Archipelago along the course of the Humboldt Current. The journey, which required 101 days, was planned by Thor Heyerdahl, Norwegian ethnologist, in an effort to prove that the early American Indians might have sailed the route to settle Polynesia. Constructed chiefly of balsa and bamboo bound together with rope and having a 15 × 18 foot canvas sail, the primitive craft was built by Heyerdahl and Herman Watzinger in the style believed to have been used during the pre-Inca period. Other members of the crew were Bengt Danielson, Torstein Raaby, Erick Hesselberg, and Knut Haugland.

Published by the
AMERICAN
ASSOCIATION
FOR THE
ADVANCEMENT
OF SCIENCE

Genetic Control of Lymphomatosis in the Fowl
F. B. Hutt and R. K. Cole

Genetic Control of Lymphomatosis in the Fowl

F. B. Hutt and R. K. Cole
Department of Poultry Husbandry, Cornell University

AVIAN LYMPHOMATOSIS IS A DISEASE characterized by the accumulation of undifferentiated lymphocytes in the nerves, in the viscera, or in the iris. If the resultant tumors are in the sciatic or brachial nerves, the leg or wing is paralyzed, and the bird is incapacitated even though otherwise in good health. In many cases nerves of the autonomic system are affected. Involvement of the ovary, liver, spleen, and kidney is common.

The disease is known—in its various forms, and by different people—as lymphomatosis, neurolymphomatosis, leukemia, the avian leucosis complex, iritis, fowl paralysis, range paralysis (because it often afflicts young birds on the rearing ranges), and big-liver disease. In Europe, it is sometimes called Marek's disease after the Hungarian pathologist who first described it in 1907. It is prevalent in many parts of the world.

Because under certain conditions lymphomatosis can be transmitted from bird to bird by inoculation with a cell-free filtrate from diseased fowls, it is generally considered to be caused by a virus. It is also spread by contact among young chickens.

The disease usually does not cause death before 6 weeks of age, but most of the susceptible birds die within 18 months of hatching. In the United States it is responsible (*4, 17*) for about 40 per cent of the high mortality now prevailing in growing stock and during the first year of life. Since the first detailed study of the disease in this country by Pappenheimer, Dunn, and Cone (*13*), many investigators have sought to discover its cause, the paths of infection, the basis for the diverse manifestations, and possible methods of control. That progress in these directions has been slow is disappointing to the poultry industry, but not surprising to anyone familiar with the difficulties besetting any study of neoplasia.

A special laboratory for research on avian lymphomatosis was established by the U. S. Department of Agriculture in 1939 at East Lansing, Michigan. The director of that laboratory, Berley Winton, has estimated (*17*) that the disease causes in this country alone an annual loss exceeding $50,000,000.

That the disease may be effectively controlled by raising the chicks from hatching to about 5 months of age in complete isolation from older birds was proved experimentally by Kennard and Chamberlin (*10*) and by Johnston and Wilson (*9*). After about 5 months of age, exposure to the disease seems not to matter. However, it is not possible for many poultrymen to provide separate attendants and equipment for their growing stock; hence, complete isolation is somewhat impracticable. Losses from lymphomatosis can still be reduced with lesser and more feasible degrees of isolation, particularly if the chicks be kept at some distance from older stock during the first few weeks after hatching, which is apparently the critical period (*6*).

The Experiment

Object. Beginning with an unselected population hatched in 1935, an experiment has been carried out at Cornell University to determine the feasibility of breeding strains of fowls resistant to lymphomatosis. It was well known then that families differed in susceptibility (*1*). The question was whether or not, by selection, genetic resistance could be established at a level high enough to constitute control of the disease in large populations that are thoroughly exposed, *i.e.* in commercial poultry flocks. Furthermore, resistant strains would be of little economic value unless superior (or satisfactory, at least) in body size, in egg production, and in egg size. It was desirable, therefore, to determine whether or not increased resistance to lymphomatosis could be attained without sacrificing these other indispensable desiderata.

Procedure. Reduced to its simplest terms, the procedure for 12 years has been (a) to have all chicks pedigreed, so that the sire and dam are known for each, (b) to ensure a natural exposure to lymphomatosis, (c) to maintain, as nearly as possible, uniform environment for all birds at every age from the egg on, and (d) to select for breeding the sires and dams whose production of the most resistant families has been proved, together with promising untested birds from those same families.

Some idea of the scope of the experiment is given by the fact that, in all selected generations after the fourth (which was hatched in 1939), from 2,100 to 2,600 females of the White Leghorn breed have been started at 6 weeks of age in tests of their viability and have been maintained under test to the age of 500 days. Complete records have been kept of their ages at commencement of laying, of their egg production, size of egg, and body size.

Every bird that died was autopsied to ascertain the cause of death. The usual thorough macroscopic examination was supplemented, when necessary, with bacteriological study, or with histological examination to determine the type of tumor involved. Most of these diagnoses were made by one of us (R.K.C.), except for a period of 46 months when he was absent in military service. During that time the examinations were made by R. F. Ball, whose assistance is gratefully acknowledged.

An essential part of the program was that no birds were culled from the flock because of small size, low egg production, or ill health. Some whole families were eliminated when the numbers of female chicks therein were too small to permit evaluation of the family's genetic potentialities, but this was done early, before the test had progressed very far. Once started, all birds were given an equal chance to demonstrate their ability to survive to 500 days of age. This does not mean that every hen still able to stagger about at that age was counted as a survivor. On the contrary, all birds then obviously sick were killed and examined, and their records were included with those of birds deceased earlier.

Full details of the procedure and management have been given in interim reports (*5–8*). Anyone comparing the data in these reports with figures in the present paper should remember that, whereas the former analyses considered a test period beginning at 160 days of age, records for the early years have now been extended to include the period from 6 weeks to 500 days.

Strains involved. The flock of White Leghorns with which the experiment was begun had been bred at Cornell University for many years for increased egg production, but no selection had been made for resistance to disease, except in so far as the least fit had eliminated themselves from the breeding pens by premature death. From this stock, three differing strains have been developed. One of these, the C Resistant line, has had no new blood introduced. Another, the K Resistant line, comes from an outcross of the original Leghorns to an entirely unrelated line in 1936. The third strain, the Susceptible line, has come from the original stock but has been bred for susceptibility to lymphomatosis.

Exposure. The birds under test have been exposed naturally, not inoculated with pathogenic material. When the experiment was started, little was known of the transmission of the disease or of the channels of infection. However, from its prevalence in this flock and in others, it was assumed that natural exposure adequate for differentiation of resistant and susceptible families (and hence for selection) was inevitable. It seemed preferable to breed for resistance to that type of infection rather than to some aberrant pathological condition that might result from artificial inoculation. This decision was fortunate, for it has subsequently been shown that genetic resistance to natural infection is not revealed by subcutaneous inoculation (*3*) or by inoculation in some manner not specified (*18*), although oral inoculation does permit differentiation of genetically resistant and susceptible stock (*3*).

To ensure adequate natural exposure, sanitation of the kind so generally advocated was practically dispensed with. Brooder houses, rearing quarters, and laying pens were cleaned when necessary but were not disinfected. Trucks, equipment, feed, and attendants moved freely from buildings housing adult birds to the rearing range. Chicks were managed during the critical first two weeks after hatching by an attendant who also cared for adult birds and whose work was so arranged that he walked many times daily from hen-pens to chick-pens.

Proof that natural exposure was ample in this experiment was provided by the high incidence of lymphomatosis among birds of the Susceptible strain. Since these were hatched along with those of the C and K Resistant strains, and mixed with them right from the incubators onward, it is obvious that all must have been equally exposed. In similar selection experiments conducted by others, failure to maintain such a susceptible strain has led to the erroneous belief that strains resistant to lymphomatosis had been quickly established when (as the experience in later years showed) they had merely been temporarily relieved from severe exposure.

Results

(A) *Neoplasms*. As is shown in Fig. 1, the selection practiced was effective in differentiating two lines highly

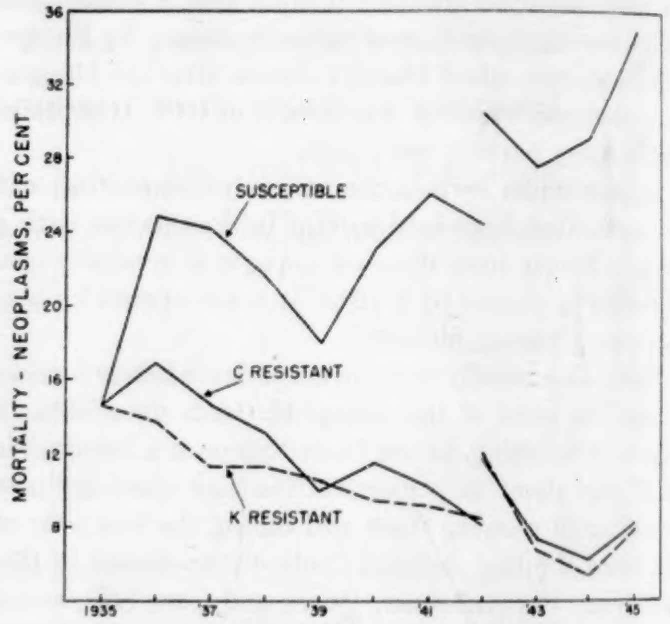

Fig. 1. Reduction in deaths from neoplasms in the C and K Resistant lines and increase in the Susceptible line during 10 generations of selection. The graphs are smoothed by using a 3-year moving average (except for terminal points). The break in 1942 is explained in the text.

resistant to neoplasms and one that was very susceptible. Of the neoplasms, 90–95 per cent were cases of lymphomatosis, but, because there was at one time disagreement among pathologists concerning the different manifestations of this disease, the all-inclusive term, neoplasms, was used from the very beginning of the experiment.

In the unselected population of 1935, 14.6 per cent died of neoplasms. In the 9th and 10th selected generations, hatched in 1944 and 1945, deaths from neoplasms in the K Resistant line were 5.4 and 7.9 per cent, respectively, and in the C Resistant line they were 6.3 and 8.3 per cent.

The reduction of neoplasms in these two lines is much more significant than the figures indicate. In 1942 it was discovered that one of the two brooder houses used was providing a much more severe exposure than the other,

possibly because of its closer proximity to adult fowls. Even though chicks were brooded in that house for only their first two weeks, they suffered over twice as many deaths from neoplasms after 5 months of age as did the chicks started in the other house (*6*). This effect is clearly shown in Fig. 1, in the data for 1942. In all three strains, the lower points for that year show, as for previous years, the deaths from neoplasms in all birds. Half of these were lightly exposed and half severely exposed. The upper points give the corresponding figures for the severely-exposed birds only. Since, in the two resistant lines, these latter figures are about 50 per cent higher than the average, it is evident that the other half of the birds was exposed rather lightly. This condition had prevailed, unknown, from the start of the experiment in 1935. After its discovery, all the chicks were started in the brooder house providing the severe exposure. That deaths from neoplasms in the two resistant lines were only 5–8 per cent in the 1944 and 1945 populations is more significant, therefore, than one might think at first glance.

Over the 10-year period of selection, deaths from neoplasms in the Susceptible line increased gradually. This is remarkable, because selection is less efficient in that line than in the others. Since the most susceptible birds die long before the normal breeding season, selection is limited to the use of the remaining birds (which are the more resistant ones) in the susceptible families. In spite of this, susceptibility was raised in the 10th generation to such a level that 34.9 per cent of the females in the Susceptible line died of neoplasms before 500 days of age. This figure was over four times those for the two resistant strains.

The foregoing data on mortality from neoplasms are based on the total populations in each line each year. Each of these groups can be divided into two classes: (a) daughters of proven sires, and (b) daughters of cockerels being tested for the first time. One might reasonably consider only the former class as the measure of the progress effected by selective breeding, as it is inevitable that some cockerels will fail to transmit in accord with the breeder's hopes and expectations. For this reason, the record for all daughters of proven sires is somewhat better than that for all daughters of cockerels and, hence, better than the averages for the strains shown in Fig. 1.

Considering now only the daughters of proven sires, selection has raised resistance to a level such that in the C and K lines deaths from neoplasms were only 2.0 and 4.4 per cent, respectively (during the 458-day test period), for the 9th generation and 6.6 and 7 per cent for the 10th generation. Exposure to the disease was severe enough to cause 34 and 38 per cent of the birds in the Susceptible line to die of neoplasms in those same years. These losses in the two resistant lines are so low as to be relatively unimportant.

(B) *Mortality from all causes.* The reduction of economic loss from mortality in the two genetically resistant strains is considerably greater than one would expect from the lowering of the death rate from neoplasms alone. In 1935, mortality from all causes during the test period was 66.8 per cent in the unselected population. In the 10th selected generation it had dropped to 22.4 per cent in the C Resistant line and 19.9 per cent in the K Resistant line (Fig. 2). These mortality rates mean a reduction of losses by about two-thirds.

Some of this reduction in mortality may properly be attributed to improvements in husbandry during the 10-year period, particularly to changes in management during the rearing period which reduced losses from coccidiosis and tapeworms. However, since total mortality in the Susceptible strain was still 53 per cent in the 10th generation, and as high as 68 per cent in the severely exposed pullets of the 7th generation (1942), it is clear that by far the greater part of the reduction of mortality in the resistant lines must be credited to breeding. Moreover, it is probable that potential deaths from lymphomatosis were actually higher in the earlier years of the experiment than the frequencies shown in Fig. 1. This is partly because many of the birds then died of intestinal parasites before an age at which they could show lymphomatosis. Moreover, some of the more obscure forms of the disease were probably not recognized in the earlier years.

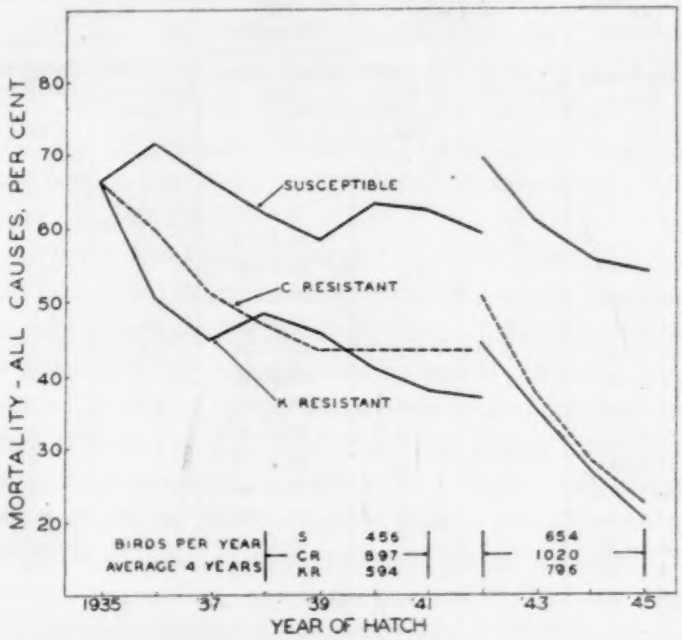

FIG. 2. Reduction in deaths from all causes in the resistant strains and maintenance of high mortality in the susceptible one. The lines show 3-year moving averages (except for terminal points).

It will be noted that between 1937 and 1942 mortality was not decreased much in the K strain and remained practically constant in the C strain. However, from 1942 onward it declined rapidly in both of these resistant lines. The reasons for this rather abrupt change in trend will be discussed later. The rise in mortality in all three lines in 1944 resulted from chronic respiratory disorders, which were unusually severe in that year.

(C) *Age at death.* Genetic differences in resistance to disease among these three strains are shown not only by the proportions that died during the test period but also

by the duration of life. Since most of the birds surviving to 500 days (all not retained for breeding) were then disposed of, a true figure for mean duration of life cannot be determined. However, among those of the 10th selected generation dying prior to 500 days of age, birds in the K and C Resistant strains lived 71 and 39 days longer, respectively, than those that died in the Susceptible strain. For these last the mean age at death was 233 days.

(D) *Differences among families.* Since the original demonstration by Asmundson and Biely (*1*) that sires differ in the degree of susceptibility to lymphomatosis transmitted to their daughters, similar differences have been commented upon by others. Some striking cases were cited in an earlier report (*7*). Without going into voluminous details, some idea of the genetic variability in this respect (which is the basis for selection) is given by the data in Table 1. This shows the distribution of 86 cockerels of the 8th, 9th, and 10th generations according to the mortality in their daughters from neoplasms and from all causes. (Lymphomatosis accounted for about 90 per cent of the neoplasms.) Only sires that had at least 30 daughters under test are included; three-quarters of them had over 40, and almost half had over 50.

TABLE 1

DISTRIBUTIONS OF 86 COCKERELS TESTED IN 1943, 1944, AND 1945 ACCORDING TO MORTALITY IN THEIR DAUGHTERS (a) FROM NEOPLASMS, AND (b) FROM ALL CAUSES, BETWEEN 42 AND 500 DAYS OF AGE

(a) Mortality from neoplasms among daughters (%)	Sires in: C and K Resistant lines (No.)	Sires in: Susceptible line (No.)	(b) Mortality from all causes among daughters (%)	Sires in: C and K Resistant lines (No.)	Sires in: Susceptible line (No.)
0	5				
0.1-3	12		5 -10	1	
3.1-6	13		10.1-15	6	
6.1-9	19	1	15.1-20	8	
9.1-12	9		20.1-25	13	
12.1-15	3	1	25.1-30	11	1
15.1-18	2	2	30.1-35	8	1
18.1-21	1	3	35.1-40	7	1
21.1-24	1	2	40.1-45	8	2
24.1-27		1	45.1-50	2	6
27.1-30		3	50.1-55		4
30.1-33		2	55.1-60	1	1
33.1-36		2	60.1-65		
36.1-39		1	65.1-70		2
39.1-42		1	70.1-75		3
42.1-45		2			
Total........	65	21		65	21

Among the 65 sires of the Resistant lines, there were 5 that lost no daughters at all from neoplasms, and this in families of which the average size was 52. At the same time, 2 males of the Susceptible line each had 44 per cent of their daughters die of neoplasms. Three of the 21 cockerels in that strain lost over 70 per cent of their daughters from all causes before 500 days of age. These cases from opposite extremes, and the range shown in Table 1, give some idea of the variation available for selection and also of the differentiation between resistant and susceptible lines brought about by that process.

When a sire's genotype has once been evaluated by an adequate progeny test, fairly consistent performance of his offspring by comparable females can be expected thereafter. This is illustrated by the records for 2 males, both of which were used for 3 consecutive years. Though mated with different females each year, deaths of daughters from neoplasms in those years were 4.3, 1.5, and 0 per cent for one sire, but 33, 47, and 41 per cent for the other. While the difference must be attributed in part to the fact that the one was mated with hens of a resistant strain and the other with hens of the Susceptible one, there can be no doubt of the consistent influence of these 2 sires on the viability of their progeny.

TABLE 2

COMPARISONS OF BODY WEIGHT, EGG WEIGHT, AND EGG PRODUCTION IN THE ORIGINAL POPULATION WITH THAT IN THE 10TH SELECTED GENERATION

Population	Adult body weight (grams)	Adult egg size (grams)	Mean eggs/bird to 500 days of age: Per hen finishing (No.)	Mean eggs/bird to 500 days of age: Per hen starting (No.)
Original unselected (1935)............	1,690	54.1	177	90
10th generation (1945)				
C Resistant strain..................	2,027	59.7	207	186
K Resistant strain..................	2,102	60.0	186	169
Susceptible strain..................	1,935	58.5	161	117

(E) *Economic characters.* To satisfy the poultryman, hens must be able not only to withstand disease but also to lay many eggs—eggs that weigh 2 ounces each or more—and to turn in at the end of the productive period a carcass large enough to be desirable for meat. While genetic resistance to lymphomatosis was being established, the two resistant strains were also steadily improved in egg production, size of egg, and body weight.

The full extent of that improvement is best shown by contrasting the performance of the 10th selected generation with that of the original population from which the resistant and susceptible lines were developed (Table 2). The original stock, though unselected with respect to viability, had been bred for high egg production for over 20 years prior to 1935 and was then considered a superior strain.

In spite of that, mean egg production in the C and K Resistant lines to 500 days for hens living to that age was increased in the decade covered in this report by 17 and 5 per cent, respectively. (The averages of 207 for one line and 186 for the other must not be belittled by comparing them with figures often cited by others for average egg production during the *first laying year*, a period that is about 55 days longer than the one used in this study.) However, the mean egg production of birds still alive at 500 days of age can be a misleading measure of value, as

It does not consider what proportion of the pullets that started were able to finish. In 1935, that proportion was only 33 per cent of the females alive at 6 weeks of age, but in 1945 it was 78 per cent in one line and 80 per cent in the other.

The better measure, therefore, is the mean egg production of the pullets that were housed in winter quarters at about 160 days (thus disregarding those that died before reaching the age of laying). This figure, sometimes called a "production index," is given in the extreme right column of Table 2 for each group of birds. The indices for the C and K Resistant lines are, respectively, 2.1 and 1.9 times that for the original population. On this basis, the productivity of these strains has been doubled during the decade of selection. In 1942, mean egg production of survivors in the C and K strains was from 10 to 50 eggs per bird higher than for pullets brought in as chicks from four leading breeders of White Leghorns and reared for these comparisons on the local premises (*8*).

Adult body weight and egg weight were recorded annually in March when the birds were about one year old. The former was increased in the decade by 20 and 24 per cent. Egg weight was raised by the 10 or 11 per cent necessary to bring it to the desired level of 58–60 grams, beyond which further increase was unwarranted.

In the Susceptible strain, body size and egg size were improved somewhat, but egg production declined. This was chiefly because high mortality left comparatively few birds for selection as breeders, but also because incipient disease in survivors still alive at 500 days of age lowered the mean production of that group.

General Considerations

In view of (a) the reduction of deaths from lymphomatosis and other neoplasms to 2–7 per cent in the offspring of proven sires and (b) the reduction of mortality from all causes by two-thirds, as well as (c) the improvement in economic characters considered in the previous paragraphs, it would seem that to the original problem one may now write *quod erat demonstrandum*. Two strains highly resistant to lymphomatosis have been developed. Both are more than satisfactory with respect to egg production, egg size, and body size.

Some say that it has taken too long to accomplish these objectives. The answer is that with the experience of the past decade the same result could now be achieved in less than 10 years. Limiting factors were (a) light exposure of half the chicks up to 1943, which lessened the effectiveness of selection in the first 6 generations, and (b) the difficulty of finding proven sires good enough to use for more than one year. These two limitations retarded progress in the K Resistant strain and completely prevented it in the C Resistant line from 1939 to 1943 (Fig. 2). After they were removed, mortality was quickly lowered in three years.

Uniformly severe exposure was ensured by starting all chicks hatched after 1942 in the brooder house that provided the more severe exposure.

The other limitation—too few proven sires—resulted from the fact that several objectives had to be considered in the selection of breeders. With a single measure of desirability, such as low mortality, half the cockerels tested will prove superior to the average. With any three additional objectives, *e.g.* egg production, body size, and efficient reproduction, half the males are again superior in each, but only about 1 in 16 is superior in all four desiderata. Study of this problem revealed that adequate tests for viability of progeny could be made with families of 30–50 daughters (*12*), these numbers being much less than had previously been considered desirable. This meant that enough daughters for a test could usually be hatched from three weeks' eggs from each breeding pen. Whereas previously each cockerel had sired chicks for the whole hatching season of 10 weeks, a "second shift" and even a "third shift" of cockerels are now used. As a result, 10 pens available for such tests now provide tests of 30 cockerels instead of 10, thus tripling the chance of having superior proven sires to use for two or three years. This has been a big factor in the rapid improvement shown from 1943 to 1945.

Other friendly critics suggest that the C and K Resistant lines differ from the Susceptible one, not in genes, but merely in lacking the causative virus. This, they suggest, is present in the Susceptible line and is regularly transmitted through the egg to each successive generation of that strain. The fact that the virus causing mammary carcinoma in mice can be transmitted in the milk of nursing mothers, or foster mothers, makes plausible the hypothesis of cytoplasmic transmission of the agent, or agents, responsible for lymphomatosis in the fowl. However, such a channel of infection has not yet been demonstrated with respect to avian lymphomatosis. To test the validity of this theory, reciprocal crosses have been made between the C and K Resistant lines and the Susceptible one. The results of these, which will be reported in detail elsewhere, provide no support for the theory, although they do not necessarily disapprove it. They show that the fate of the chick depends as much upon the genes received from the sire as on genes and any cytoplasmic agent transmitted by the dam.

In similar studies, Taylor, *et al.* (*15*) found it easier to lower resistance by selection than to raise it. Some progress was apparently made toward establishing resistance in the first three generations, but this was later lost. From their data it seems possible that insufficient numbers of proven sires may have been a limiting factor, as it was in the Cornell experiments until the "double shifts" and "triple shifts" were introduced. At the Regional Poultry Laboratory, East Lansing, deaths in the 6th selected generation from lymphomatosis alone were already up to 11.4 per cent in their resistant stock by 300 days of age and

about twice as high in the susceptible lines (*19*). However, since investigators there are attempting to combine breeding for resistance to lymphomatosis with a program of close inbreeding, it is not surprising that progress has been slow.

Utilization of Resistant Stock

The object of this experiment was to determine the feasibility of controlling genetically the most serious disease besetting the poultry industry and of improving productivity at the same time. We feel that this has been done. The extent to which poultry breeders not supported by public funds would be justified in undertaking the same sort of work remains to be determined. The desirability of maintaining a susceptible line to ensure exposure may be a deterrent, as such a stock can hardly be profitable. At the same time, more and more breeders and hatcherymen are recognizing that stock sold from flocks not exposed to disease may be the cause of intense dissatisfaction when it is so exposed in the hands of customers. As one of them (*16*) expressed it: "I am convinced . . . that no breeder can afford to have a disease-free farm, or even a relatively disease-free farm." So far as lymphomatosis is concerned, the disease is enzoötic and ubiquitous. Exposure is almost inevitable except in the smaller and more isolated flocks. Experience may show that breeders can develop resistant stock by exposing only enough of their chicks each year to provide tests of sires, while keeping the major part of the birds isolated, in so far as this is possible, and hence comparatively free from disease so that their productivity will not be impaired.

Even without any deliberate exposure to disease, poultrymen can raise the viability of their stock by selective breeding. While this has been demonstrated by Sturkie (*14*), by Bostian and Dearstyne (*2*), and by others, one must be prepared to find such stock highly susceptible to lymphomatosis when conditions are right for its reappearance (*11*).

Another possibility is that resistant strains may be developed at the agricultural colleges and experiment stations and distributed as have been the rust-resistant varieties of wheat and disease-resistant strains of other plants. However, since fowls are dioecious organisms, unless the strains resistant to lymphomatosis are inbred to the stage of almost complete homozygosity (which is unlikely if they are to be economically profitable), continued selection would be necessary to maintain the original resistance.

It may prove more practicable to utilize the resistant strains by sending out their desirable genes in thousands of cockerels to head flocks that supply the hatcheries. Such tests as we have been able to make thus far indicate that cockerels of the resistant stock, when mated with birds unselected for resistance to lymphomatosis, induce a remarkable increase in viability and even in egg production. If these results are substantiated in further tests, it would seem desirable to continue selection, and to have "multipliers" produce sufficiently large numbers of the resistant birds to supply the hatcheries with thousands of males. Since about 90 per cent of the country's chicks are now produced by commercial hatcheries, such a scheme would provide the maximum possible utilization of the genetic resistance that has been established by breeding.

References

1. Asmundson, V. S., and Biely, J. *Canad. J. Res.*, 1932, **6**, 171.
2. Bostian, C. H., and Dearstyne, R. S. *N. C. agric. exp. Sta. tech. Bull. 79*, 1944.
3. Heisdorf, A. J., Brewer, N. R., and Lamoreux, W. F. *Poultry Sci.*, 1947, **26**, 67.
4. Hutt, F. B. *Amer. Nat.*, 1938, **72**, 268.
5. Hutt, F. B., Bruckner, J. H., and Cole, R. K. *Proc. 7th World's Poultry Congr.* (Cleveland), 1939, 49.
6. Hutt, F. B., Cole, R. K., Ball, M., Bruckner, J. H., and Ball, R. F. *Poultry Sci.*, 1944, **23**, 396.
7. Hutt, F. B., Cole, R. K., and Bruckner, J. H. *Poultry Sci.*, 1941, **20**, 514.
8. Hutt, F. B., Cole, R. K., and Bruckner, J. H. *Poultry Sci.*, 1945, **24**, 564.
9. Johnston, J. W., and Wilson, J. E. *Vet. J.*, 1937, **93**, 13.
10. Kennard, D. C., and Chamberlin, V. D. *Ohio agric. exp. Sta. Bimonthly Bull.*, 1936, **21**, 63.
11. Lampman, C. E., and Holm, G. C. *Idaho agric. exp. Sta. Bull. 251*, 1943, 22.
12. Mueller, C. D., and Hutt, F. B. *Poultry Sci.*, 1946, **25**, 246.
13. Pappenheimer, A. L., Dunn, L. C., and Cone, V. *Storrs (Conn.) agric. exp. Sta. Bull. 143*, 1926.
14. Sturkie, P. D. *Poultry Sci.*, 1943, **22**, 155.
15. Taylor, L. W., Lerner, I. M., DeOme, K. B., and Beach, J. R. *Poultry Sci.*, 1943, **22**, 339.
16. Wallace, H. B. *Nat. Poultry Dig.*, 1946, **5**, 461.
17. U. S. Department of Agriculture (Regional Poultry Research Laboratory, East Lansing, Michigan). *4th ann. Rep.*, 1943, 3.
18. U. S. Department of Agriculture (Regional Poultry Research Laboratory, East Lansing, Michigan). *5th ann. Rep.*, 1944, 18.
19. U. S. Department of Agriculture (Regional Poultry Research Laboratory, East Lansing, Michigan). *7th ann. Rep.*, 1946, 3.

Toward a National Science Policy?

Study Group, Washington Association of Scientists

Washington, D. C.

AFTER TWO YEARS IN GESTATION A National Science Foundation Bill emerged from the 80th Congress, only to be vetoed by the President (*Science*, September 12, pp. 236–239). This outcome is undoubtedly puzzling to those who have followed the course of the legislation and are aware of the almost unanimous support for the establishment of a strong national science policy. The paradox exists, however, only when viewed from a distance. Close analysis of the bills introduced into the 79th and 80th Congresses (*Science*, December 27, 1946, pp. 614–619), of the Congressional hearings and debates on these bills, and of the President's veto message and the relevant sections of the recent reports of John R. Steelman (*1*), special assistant to the President, and Attorney General Clark (*2*) shows a sharp cleavage between two opposed philosophies of the relation of science to government and society. From the introduction in the 79th Congress of the original Kilgore and Magnuson Bills, which were based on two sharply divergent conceptions of the nature and purposes of the proposed Foundation, down to the Presidential veto of S. 526, the fundamental dichotomy has persisted and prevented successful completion of the legislation.

In the most general terms, the conflicting philosophies appear to be these. That of the original Kilgore Bill, concurred in by the President and his advisers as well as by many scientists, is based on the premise that science is a national resource, that its raw material is the Nation's scientific manpower, and that, as a vital national resource, its furtherance should be entrusted to an authority directly responsible to the elected representatives of the people—the Congress and the President. The proponents of this philosophy place primary emphasis upon long-range planning for the whole field of science to ensure the development of scientific potential on the widest possible basis throughout the country. They seek guarantees which will deny to special interests a disproportionate influence in formulation of Foundation policy, or disproportionate benefits from its activities. They insist upon a patent policy which will permit free public access to discoveries made with public funds.

The opposing philosophy, embodied in the original Magnuson Bill and, in even more extreme form, in the recently vetoed Smith Bill, regards science as an auxiliary to the development of industry, medicine, and the national defense; it places complete confidence in the existing organizations and facilities for research and believes that these organizations should further the development of science with a minimum of control by the elected representatives of the people. It would thus simply expand scientific activity in the country by enlarging the existing structure, concentrating support in well-tested organizations and centers if results may be thus more effectively attained. It would place control of the Foundation in the hands of recognized leaders in science, industry, and national defense, insulating it from the people's representatives in the interests of security and immediate efficiency.

It is clear that these differences between the two opposed points of view are fundamental and underlie the swirl of controversy which has gone on about more specific issues, e.g. form of administration, inclusion of social sciences, geographic distribution, etc. The basic issue is none other than the proper role of the Federal Government in regulating those areas of our national life which are intimately related to the public welfare and security, in this instance the shape and scope of science. It is not surprising, therefore, to find that groups, organizations, and individuals have lined up on the National Science Foundation very much as they have on atomic energy, national health insurance, Federal support of housing, and similar issues. Science, with its present budget of approximately $1,000,000,000 and a recommended budget (Steelman report) of 1 per cent of the national income, can apparently no longer remain out of the political arena. Issues of fundamental national policy are involved, issues important enough to produce

This analysis of the present status of national science legislation, up to and including the Presidential veto and the subsequent Steelman report, was made by a Study group of the Washington Association of Scientists (a branch of the Federation of American Scientists), consisting of C. Grobstein (chairman), J. M. Conly, I. Feister, L. B. Heilprin, H. Olken, F. J. Pratt, J. W. Rowen, I. Schocken, G. R. Silbiger, R. D. Steiehler, F. J. Weiss, and L. A. Wood. The group had the advantage of being on the spot during consideration of the legislation by Congress, and obtained first-hand information through attendance at committee hearings and floor debate and through interviews and correspondence with interested legislators. Their analysis reveals the basic conflict which has so far prevented the passage of a National Science Foundation Bill.

an impasse between the executive and legislative branches of the Government, as expressed in the recent Presidential veto.

In actual fact, the area of agreement between the contending philosophies is limited to the most general features of the legislation. Nearly all parties concur that some Federal financing of science is required, that the responsible agency should be in civilian hands, and that major emphasis should be given to fundamental or basic research, albeit the exact definition of the latter has remained somewhat hazy. The necessity for increased training of scientific manpower also is generally supported, as well as the importance of coordinating the scientific work of Federal agencies and of encouraging international exchange of scientific information and personnel within the limits of national security.

But beyond these most general features the deep cleavage appears, and the debate becomes bitter. The form of administration of the Foundation has been a major storm center. To many observers this has seemed unfortunate since, it has been said, in the final analysis the success of an organization depends upon its personnel rather than its organization chart. But the opposing schools of thought have sensed in this issue the crux of their entire difference. The Magnuson-Smith school has sought to design the Foundation so as to effect a minimum of change in the existing structure of science. They have tried to erect an administrative barrier between the science agency and the ordinary instruments of Federal authority—a barrier, in other words, which would be permeable to the Federal dollar but impermeable to the virus of Federal control. They have placed final administrative authority in an unsalaried board consisting of scientists and other authorities serving on a part-time basis. In its most extreme form, the original Smith Bill introduced into the Senate of the 80th Congress, this board was to consist of 48 individuals. It was to elect from its own membership an executive committee of 9, which would in turn select a director, the actual administrative head of the Foundation. It was this complex structure which was denounced by the President in his veto message as implying "a distinct lack of faith in democratic processes" and offering the danger that "it would impede rather than promote the Government's efforts to encourage scientific research."

On the other hand, the Kilgore-Administration school believes that science has grown to such stature, and is so important for the national well-being, that its management can be left neither to chance nor in the hands of a small group of private citizens, serving part-time, no matter how well qualified or well intentioned they may be. Moreover, they feel that an activity which is fundamentally geared into the main drive-shafts of our economic and social life cannot be left free from the normal processes of democratic political control. Recognizing the need for protection of the freedoms of the individual investigator from irresponsible political meddling, they nevertheless would firmly integrate the National Science Foundation in the Federal governmental structure. Thus, they would place the direction of the Foundation in a single individual, or at most a small, full-time commission, appointed by the President and confirmed by the Senate, and fully responsible to these elected representatives of the people. They would retain the advantages of a larger part-time board by establishing it in a purely advisory capacity.

Thus, the quarrel over administration is essentially one over the nature of the Foundation. The former view would make of it a virtually autonomous agency, Federal only in its financing, quasi-governmental in structure. The latter would make of it a truly Federal agency integrated in the governmental structure and capable of closely coordinated action with agencies responsible for other aspects of the national life—education, industry, agriculture, defense.

No less sharp has been the cleavage over patent policy. The Magnuson-Smith school seeks to avoid the problem by directing the Foundation to remain within the limits of existing patent policies and practices, executing its contracts "in a manner calculated to protect the public interest and the equities of the individual or organization" (S. 526) involved. The opposing school argues that new problems have been created by the wide-spread support of research by Federal funds, and that existing patent practices and policies are inadequate both to protect free scientific publication and to insure the full exploitation for the benefit of the public of discoveries financed by public funds. They advocate, with certain safeguards, the free availability or the free dedication of all patentable discoveries arising from government-financed research.

Again, in the matter of distribution of funds in support of research, the basic conflict is revealed with the Magnuson-Smith school arguing against any specific directive on the basis of population and geography, on the ground that such mandatory distribution would hamper the Foundation and constitute a "pork-barrel" for all future Congresses. The Kilgore-Administration school argues that the widest possible distribution is required in order to stimulate the growth of science throughout the Nation, and mandatory provision of some kind is the only guarantee against the natural tendency toward centralization of support in already well-established institutions and organizations.

Thus, after nearly three years of debate the issues remain undecided, the contending philosophies unreconciled. It is impossible to predict at this moment what new action may be expected when the 80th Congress reconvenes. The probability of passage of politically disinterested legislation by a Congress in a presidential election year is notoriously low. Meanwhile, new trends are developing, and patterns are being established in the

relationship between science and the remainder of society. In spite of almost universal desire for control of science policy by civilians, the absence of a National Science Foundation is establishing control more firmly in military hands. It is widely conceded that we have been weakest in fundamental research, and that strong measures are required to strengthen this aspect of our science. But the present tendency appears to be strongly in the opposite direction, with available funds for research bearing on industrial, military, and health problems enormously overbalancing those available for research having no obvious immediate practical importance. We are in grave danger that our universities will become adjuncts of, and recruiting grounds for, the laboratories devoted to application. Here again goes the goose that lays the golden egg.

As scientists we cannot escape our share of responsibility for the present hazardous state. Congressmen who were interviewed displayed a flattering interest in the views of scientists and their organizations on national science legislation, but many confessed their lack of knowledge of details of the legislation and stated that they had had little advice from home to guide them. One remarked ruefully that, if this were a labor issue, he would have heard from every labor leader in his district. He was forced to conclude that scientists were not very much interested one way or the other.

It must, indeed, be regarded as amazing that individual scientists have made so little effort to influence the legislation, considering the inescapable effect on science and on their personal future that the establishment of a National Science Foundation must have. National scientific organizations banded together in the Inter-Society Committee, and their representatives participated in committee hearings. But when the chips were down and the individual legislators were making up their mind on how to vote, there was very little pressure of the kind that counts—communications from individuals, colleges, institutes, societies, and academies in the *home districts*.

With the formation of the Inter-Society Committee at Boston in December 1946, was there a complacent tendency on the part of individual scientists to overestimate the potential effectiveness of this organization, with a resulting decline in other types of activity? Have we misread the tactical picture assuming the issue to be National Science Foundation, yes or no? The Inter-Society Committee spoke strongly and effectively in favor of the establishment of a National Science Foundation, but was much less clear in its stand on the specific questions which lay at the heart of the controversy and prevented a successful outcome for the bill. Do recent events indicate that the issue is not whether we shall have a Foundation, but, rather, what kind of a Foundation we shall have? What indeed is the proper role of the Federal Government in the support, planning, and direction of science?

As the time for a new Congressional session draws near these are the questions which occupy the minds of observers here in Washington. National science policy will be decided with or without the participation of scientists. But the wisdom of the decisions will in large measure depend upon the forcefulness with which scientists on both sides of the controversy express their considered judgments, both publicly and to their representatives, *now*.

References

1. *Science and public policy*. Vol. 1: *A program for the nation*. (A report to the President by John R. Steelman, chairman, The President's Scientific Research Board, August 27, 1947.) For sale by Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C. $.20.
2. *Investigation of government patent practices and policies*. Vol. 1: *Final report proper*. (Report and recommendations of the Attorney General to the President.) For sale by Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C. $.35.

NEWS *and Notes*

Charles J. Vitaliano, formerly with the Non-Metals Section of the U. S. Geological Survey, is now associate professor of field geology at Indiana University. Dr. Vitaliano, who partly replaces **Clyde A. Malott,** who retired on June 30, will develop the new four-year curriculum of training in field geology.

John R. Paul, Yale University School of Medicine, will deliver the Gehrmann Lectures at the Chicago Professional Colleges of the University of Illinois on November 19–20. Dr. Paul will speak on "Poliomyelitis—Certain Epidemiological Aspects" and "Poliomyelitis—The Clinical Disease" at 1:00 P.M. on each day in Room 221, 1853 West Polk Street.

Linus Pauling and **George W. Beadle,** both of California Institute of Technology and both Silliman Lecturers at the centennial celebration of the Sheffield Scientific School, Yale University, received honorary D.Sc. degrees from Charles Seymour, Yale president, on October 17.

Haven Emerson, professor emeritus, School of Public Health, Columbia University, will deliver an address on "Some Factors in Preventing Disease" on October 28 at 4:00 P.M. in the Hunter College Playhouse, 695 Park Avenue, New York City. His address inaugurates the annual Margaret Barclay Wilson Memorial Lecture series, established by the Department of Physiology, Health, and Hygiene, Hunter College, as a tribute to the memory of its first chairman.

William E. Wilson, of the Biology Department, Muskingum College, New Concord, Ohio, has been appointed assistant professor of botany at Miami University, Oxford, Ohio.

Harry J. Fuller, professor of botany, University of Illinois, spent a part of the summer in Peru and Bolivia, collecting and photographing the plants of those two regions.

W. E. Caldwell and **J. P. Mehlig** have been promoted to the rank of professor, **Ralph W. Spitzer** to associate professor, and **Allen B. Scott** to assistant professor in the Chemistry Department of Oregon State College, Corvallis.

George Polya, professor of mathematics, Stanford University, and **Hermann Weyl,** Institute for Advanced Study, Princeton, New Jersey, have been elected foreign members of the French Academy of Sciences. The only other living American mathematician to have received this distinction is **L. E. Dickson,** professor emeritus, University of Chicago.

Donald S. Farner, formerly of the University of Wisconsin, has been appointed associate professor of physiology, State College of Washington, Pullman, where he will have charge of instruction in physiology in the College of Sciences and Arts.

John Emsley Funnel, research ceramist, Products Development Department, Corning Glass Works, has been appointed ceramic engineer and economic geologist, Midwest Research Institute, Kansas City, Missouri.

Catherine Personius, chairman, Department of Food and Nutrition, College of Home Economics, Cornell University, has been appointed assistant director, Cornell University Agricultural Experiment Station.

R. C. Gutschick, formerly geologist, Gulf Oil Corporation, Oklahoma City, has been appointed assistant professor of geology, Division of Geology, University of Notre Dame.

Paul M. Gross, head, Department of Chemistry, and chairman, Research Council, Duke University, has been appointed dean of the Graduate School, succeeding **Calvin B. Hoover,** who will continue as chairman, Department of Economics.

William E. Feist, formerly development engineer, Cambridge Instrument Company, Inc., Ossining, New York, has been appointed assistant professor of electrical engineering, University of Missouri, School of Mines and Metallurgy, Department of Electrical Engineering, Rolla, Missouri.

Hermann J. Muller, professor of zoology, Indiana University, lectured on the topic, "The Production and Avoidance of Mutations," at a dinner given in his honor by the Purdue Chapters of the American Society of Plant Physiologists, Society of the Sigma Xi, and the Purdue Biological Society, October 20, 1947.

Visitors to U.S.

R. C. Evans, Crystallographic Laboratory, University of Cambridge, England, will visit this country at the invitation of the American Society for X-Ray and Electron Diffraction to see recent American developments in the field of crystallographic equipment. Dr. Evans is technical editor of the new publication, *Acta Crystallographica.*

J. N. Van Niekerk, National Physical Laboratory, Council for Scientific and Industrial Research, South Africa, is now visiting the United States and expects to remain here until January 1948.

F. R. N. Nabarro, University of Bristol, is coming to the United States to inspect metallurgical work in this country. Dr. Nabarro, whose visit is sponsored by the Royal Society and the British Iron and Steel Research Association, plans to remain here about three months.

W. J. Lutjeharms, professor of botany, University College of the Orange Free State, arrived in this country in August on a grant from the South African Council for Scientific and Industrial Research.

A. C. Riddle, physical chemist, Building Research Station, Department of Scientific and Industrial Research, England, is now in the United States doing chemical liaison work for the United Kingdom Scientific Mission.

F. X. Laubscher, senior research officer, Department of Agriculture, South Africa, and in charge of plant breeding work, College of Agriculture, Potchefstroom, is in this country to investigate fiber production and to visit experiment stations where corn-breeding is being done. Mr. Laubscher leaves for New Zealand and Australia at the end of this month.

Werner Nowacki, University of Berne, Switzerland, who lectured on "The Distribution of Crystal Structures Among the Space Groups, and the Symmetry

inciples of Organic Crystals," September 22 at Alabama Polytechnic Institute, is now returning to Switzerland after visiting many American laboratories and working for 6 months with the crystal analysis group, under the direction of Linus Pauling, at the California Institute of Technology.

Grants and Awards

The Laurentian Hormone Conference on September 12 conferred the Roche-Organon Awards on Fuller Albright, of Boston, Dwight J. Ingle, of Kalamazoo, and R. D. H. Heard, of Montreal. The Awards were of $500 each, instead of the amounts erroneously announced in *Science*, September 5.

National Dog Week, Inc., 424 Madison Avenue, New York City, is now considering candidates for the $2,000 National Dog Week research award, which will be presented early in 1948. The award will be given for the most outstanding contribution to the welfare of dogs during the year 1947. Candidates may submit their reports to the National Dog Week or be nominated by a friend. The jury on awards is made up of C. A. Elvehjem, dean, Graduate School, University of Wisconsin; A. C. Ivy, vice-president, Chicago Professional Colleges, University of Illinois; W. A. Young, Anti-Cruelty Society, Chicago; James H. Steele, chief, Veterinary Public Health Section, U. S. Public Health Service, Washington, D. C.; and Carl F. Schlotthauer, Mayo Foundation, Rochester, Minnesota.

Warren A. Marrison, of the technical staff, Bell Telephone Laboratories, has been awarded the British Horological Institute's Gold Medal for 1947 in recognition of pioneer researches in the development of the quartz crystal clock. The medal will be presented by Sir Harold Spencer Jones, Astronomer Royal and president, British Horological Institute, at its 89th annual general meeting in London, October 29. On November 6, Mr. Marrison will lecture before the Institute on "The Evolution of the Quartz Crystal Clock." The quartz crystal clock, as now developed, has become the world's most accurate timekeeper. Its rate is regulated by a control unit made from quartz crystal, the stability of which exceeds that of all other control devices previously used. A clock of this type, located at Bell Telephone Laboratories headquarters, New York City, is accurate to well within a second a year.

The American Pharmaceutical Manufacturers' Association will present its 1947 scientific award to the American Medical Association at its midyear meeting to be held December 15–17 in New York City. This award is made annually, on nomination by a scientific advisory committee, for a fundamental research contribution to public health in the field of drug therapy. Previous recipients have been the Mayo Foundation for Medical Research (1946); the Rockefeller Institute for Medical Research (1945); the National Research Council (1944); and Alexander Fleming and Howard W. Florey (1943).

Colleges and Universities

Iowa State College has recently awarded 6 Master of Science degrees in agricultural climatology, a new course which has been worked out as a cooperative venture between the College and the U. S. Weather Bureau. Plans for the course, leading to M.S. and Ph.D. degrees in agricultural climatology, were initiated in the fall of 1944 by H. C. S. Thom, U. S. Weather Bureau, in cooperation with R. E. Buchanan, dean, Graduate School, and director, Iowa Agricultural Experiment Station, and W. H. Pierre, chairman, Section in Agronomy, Agricultural Experiment Station. Five of the graduating students were assigned to positions in the Weather Bureau, while the sixth will return to Brazil to resume teaching in the Minas Gerais State College of Agriculture.

The Ohio State University has recently created the Julius F. Stone Research Professorship in Physics in memory of the late Julius F. Stone, chairman emeritus, Board of Trustees, for more than 20 years until his death, July 25, 1947. The new professorship, which has not yet been filled, will have special reference to nuclear physics, the fundamental relationships between matter and energy, and the biological and medical applications of radiations.

The Department of Psychology, University of New Mexico, has three recent additions to its staff. **James C. Coleman,** University of Kansas, and **Morton J. Keston,** University of Minnesota, have been appointed assistant professors; and **David T. Benedetti,** graduate assistant, University of New Mexico, has been named instructor.

The University of Tennessee has added the following new members to its Department of Botany: **Russell B. Stevens,** Alabama Polytechnic Institute; **J. Herbert Taylor,** University of Oklahoma; **Lowell F. Bailey,** TVA Forestry Laboratory; **Shirley Hoover Taylor,** University of Oklahoma; **Frederick H. Norris,** Ohio State University; and **Kenneth A. Wagner,** University of Michigan.

The University of Minnesota has made several recent changes in the staff of its Department of Botany. **A. Orville Dahl** has been named chairman of the Department succeeding **Ernst C. Abbe,** who has completed his three-year term in that office, and who will continue as professor of botany. **Donald B. Lawrence** has been promoted from assistant to associate professor and **R. M. Tryon, Jr.,** from lecturer to assistant professor and curator of the Herbarium. **Allan H. Brown,** University of Chicago, **Harlan P. Banks,** Acadia University, and **Albert W. Frenkel,** University of Rochester, have been appointed assistant professors, and **Gerald B. Ownbey,** Missouri Botanical Gardens, instructor.

The University of Oregon has recently made several faculty appointments and promotions. In the Department of Anthropology, **Daniel S. Davidson,** formerly of the University of Pennsylvania, has been appointed associate professor and assistant curator of Anthropology, and **Robert F. Spencer,** Reed College, has been appointed assistant professor. **Eugene P. Cooper,** formerly research physicist, Naval Ordnance Test Station, Inyokern, and **Frederick W. Paul,** Institute of Optics, University of Rochester, have joined the Department of Physics as associate professors. **Clarence W. Clancy** has been promoted to associate professor, and **I. M. Newell** to assistant professor, in the Department of Biology.

The Department of Biology, University of Colorado, has added **Edwin R. Helwig,** Department of Zoology, University of Pennsylvania, and **T. Paul Maslin,** formerly of Colorado State A. & M. College, as assistant professors.

Santa Barbara College, University of California, has made several recent changes in its Department of Biological Sciences. **Mary M. Erickson,** assistant

professor of biology, has been promoted to associate professor of zoology, and **James L. Walters** and **Roscoe C. Main** have been appointed instructors in botany and zoology, respectively.

The University of Texas has made the following appointments in its Department of Chemistry: **L. O. Morgan,** formerly with the Manhattan Project at the Universities of Chicago and California and co-discoverer of the element Americium, has been appointed assistant professor; **Royston M. Roberts,** recently of the University of California at Los Angeles, has been appointed assistant professor; and **Frank Field,** Duke University, has been appointed instructor.

At the College of Medicine, University of Nebraska, there have been a number of faculty changes. **Harold E. Eggers,** chairman, Department of Pathology and Bacteriology, has retired; **J. P. Tollman** has been appointed professor of clinical pathology and chairman, Department of Clinical Pathology and Bacteriology; **John R. Schenken,** formerly head, Department of Pathology, Louisiana State University, and pathologist, Nebraska Methodist Hospital, Omaha, has been appointed professor of pathology and acting chairman, Department of Gross and Microscopic Pathology; **Pliney Allen,** formerly associated with Louisiana State University and Pratt Diagnostic Hospital, Boston, has been appointed assistant professor of pathology, and pathologist, Immanuel Hospital, Omaha; and **Robert M. Allen,** University of Minnesota, has been appointed assistant professor of bacteriology.

Meetings

The Fifth Annual Pittsburgh Conference on X-Ray and Electron Diffraction, sponsored by the Mellon Institute and the University of Pittsburgh, will be held November 7–8 at the Mellon Institute, Pittsburgh, Pennsylvania. The first session, beginning at 9:40 A.M. Friday, will be a symposium on "Interstitial Compounds and General Papers." At 2:00 P.M. Friday, the subject of the session will be "X-Ray and Electron Diffraction Studies at High Temperatures." A dinner will be held at the Faculty Club, University of Pittsburgh, on Friday evening, after which Sterling B. Hendricks, principal chemist, U. S. Department of Agriculture, will speak on "Crystal Structure and Lattice Termination in Clays and Related Products" at the Mellon Institute Auditorium. The Saturday morning session will be a symposium on "Lattice Imperfections and General Papers." Saturday afternoon's symposium will be on the topic, "Geiger-Counter X-Ray Spectrometer Studies."

The Alabama Polytechnic Institute, in cooperation with the Oak Ridge Institute of Nuclear Studies, will hold a three-day conference on "The Use of Radioactive Isotopes in Agricultural Research" on December 18–20, at Auburn, Alabama. The program will include: "Techniques of Tagged Atom Research," W. A. Arnold, Clinton Laboratories, Oak Ridge; "Radioactivity and Radioisotopes," Fred Allison, Alabama Polytechnic Institute; "Isotopes Available for Research," Paul C. Aebersold, AEC, Oak Ridge; "The Measurements of Radiations by Various Methods," Paul W. McDaniel, AEC, Washington, D. C.; "Protective Precautions in the Handling of Radioactive Materials," G. William Morgan, AEC, Oak Ridge; "Contributions of the Atomic Energy Commission to Agricultural Research," John C. Franklin, AEC, Oak Ridge; "Agricultural Research With Radiophosphorus," S. B. Hendricks, Bureau of Plant Industry, Beltsville, Maryland; "Studies of Chlorosis Using Radioactive Phosphorus and Iron," Orlin Biddulph, State College of Washington; "Agricultural Research With Radioactive Sulfur and Arsenic," M. D. Thomas, American Smelting and Refining Company, Salt Lake City; Demonstration of a typical tracer experiment, Wendell C. Peacock, Clinton Laboratories, Oak Ridge; "Atomic Energy Radiations and Plant Nutations," S. J. Sadler, University of Missouri; "Use of C^{14} for Tracer Research," Howard B. Skipper, Southern Research Institute, Birmingham, Alabama; "Nutrition Studies With Radiocobalt," J. G. Davis, University of Florida; and topics to be selected, by G. Harold Copp, University of California. The attendance, through special invitations, will consist of representatives largely from the southeastern states.

The American Association of Physics Teachers will hold its 17th annual meeting December 29–31 at the University of Chicago in cooperation with the AAAS and the American Physical Society. The 7th Richtmyer Memorial Lecture will be given at the meeting, and the Oersted medal will be presented. Those desiring to present papers must submit titles and abstracts, typewritten double spaced and in triplicate, by November 10 to the program chairman, J. W. Buchta, Department of Physics, University of Minnesota, Minneapolis 14, Minnesota. Programs of this meeting will be mailed to all Association members soon after November 10. Members who have business for the Executive Committee, which will meet in December, should present it in writing to the secretary, C. J. Overbeck, Northwestern University, before December 1.

The American Mathematical Society held its 53rd summer meeting at Yale University September 2–5, in conjunction with meetings of the Mathematical Association of America and the Institute of Mathematical Statistics. T. R. Hollcroft, associate secretary, reports that over 700 persons attended, including 443 members of the Society. Oscar Zariski, Harvard University, delivered the four Colloquium Lectures on "Abstract Algebraic Geometry," and S. S. Wilks, Princeton University, gave an address entitled "Sampling Theory of Order Statistics." A total of 149 research papers were presented, 73 in person and 76 by title. At the meeting of the Council of the Society, Tuesday evening, and also at a business meeting of the Society, it was announced that the late Mrs. John Irwin Hutchinson had made a bequest of $1,000 to the Society in memory of her husband, professor of mathematics at Cornell University who had been associated with the University from 1894 until 1935, one of the first cooperating editors of the *Transactions* of the Society, and vice-president in 1910.

The Rocky Mountain Laboratory, Hamilton, Montana, was host to the International Great Plains Conference of Entomologists, August 11–13, and to the International Northwestern Conference on Diseases of Nature Communicable to Man, August 13–16. Participants present were from 13 states and the District of Columbia, four Canadian provinces, Spain, Holland, India, and Mexico. The programs included tours of the Laboratory, visits to Montana State University, and lectures and discussions of interest to those in attendance.

NRC News

"Personnel and Training Problems Created by the Recent Growth of Applied Statistics in the United States" is the title of a report recently prepared by the Committee on Applied Mathematical Statistics of the NRC. Chairman of the group is Luther P. Eisenhart, former chairman, Division of Physical Sciences, NRC, and the secretary is Samuel S. Wilks, professor of mathematical statistics, Princeton University. Other members include: Chester I. Bliss, Connecticut Agricultural Experiment Station; Edward U. Condon, National Bureau of Standards; Harold O. Gulliksen, Princeton University; Lowell J. Reed, Johns Hopkins University; Charles F. Roos, The Econometric Institute, Inc.; Walter A. Shewhart, Bell Telephone Laboratories; Hugh M. Smallwood, U. S. Rubber Company; and Frederick F. Stephen, Cornell University.

As a simple indication of growth of interest in statistical methods, the Committee describes the formation and recent growth of statistical organizations. The American Statistical Association, founded more than 100 years ago had a membership of 1,700 in 1935. By the end of 1946 it had nearly 4,000 members. The Institute of Mathematical Statistics, formed in 1935 to promote the development of statistical theory, had 900 members by the end of 1946. The Econometric Society, with a membership of more than 750, was organized in 1930 to promote the application of mathematics and statistical methods in economics. The Psychometric Society, a similar organization for psychology, was organized in 1935 and now has more than 200 members. The Biometrics Section of the American Statistical Association, formed in 1938 for sponsoring similar work in the biological sciences, now has more than 1,100 members. The most recent statistical organization is the American Society for Quality Control, which is concerned with applications of statistical methods in industry. Organized early in 1946, it now has approximately 2 000 members, mostly engineers. There are other organizations with considerable interest in statistical methods such as the American Marketing Association, American Public Health Association, American Sociological Society, and Population Association of America.

According to the report, there is a heavy demand for both academic and nonacademic statistical personnel. Nonacademic fields which account for most of the recent growth of interest in statistical methods are: (1) industrial statistical control (in quality control, research, and development), (2) research in the biological sciences, (3) collection and analyses of government statistics, (4) market research and commercial surveys, and (5) psychological testing. Each is discussed in some detail. Demands are continuing and increasing for statistical personnel in some of the older fields such as finances and taxes, labor and employment, prices and production. Demands for more statistical training for social scientists are increasing.

The Committee made an inquiry among 30 leading authorities at 27 universities in mathematical and applied statistics as to requests received for statistical personnel for a period of approximately 6 months after the end of the war. These authorities reported a total of 135 requests for personnel for academic positions in mathematical and applied statistics ranging from instructorships to full professorships. No attempt was made to have each respondent identify each request so as to eliminate duplication. But one person reported that he had received requests from 21 college and university mathematics departments for Ph.D.'s in mathematical statistics. Another reported 12 requests for Ph.D.'s in agronomy with minors in statistics. Ninety requests from government agencies and 140 from industry were reported. The training requirements for these requests ranged from B.A.'s to Ph.D.'s in mathematical and applied statistics.

At least a rough comparison may be made between demands for personnel in mathematics, physics, and statistics. As of December 31, 1945, the National Roster of Scientific and Specialized Personnel had registrations of 9,972, 9,608, and 2,018 in mathematics, physics, and statistics, respectively. From September 1, 1945, to May 31, 1946, the numbers of requests for personnel in these three fields per 1,000 persons registered were 4.4, 23.9, and 30.7, respectively.

More than a third of the report is devoted to problems of education and training in statistics, which were discussed at both the undergraduate and the graduate level. It was stated that although substantial progress had been made in the teaching of statistics at the graduate level in a number of universities, it was still inadequate to meet the growing demands for statistical personnel. The Committee charged that the teaching of statistics at the undergraduate level was still in a very chaotic condition. Graduate teaching in mathematical statistics is more standardized than that in applied statistics. Basic requirements in mathematics for graduate training in mathematical statistics were listed as follows: real and complex variables, linear and quadratic forms, matrix algebra, n-dimensional Euclidean geometry, measure and integration theory. The courses are essential for the theory of probability which is the foundation for courses in advanced mathematical statistics covering distribution theory, estimation theory, testing of statistical hypotheses, and multivariate statistical theory.

Of the 27 universities included in the inquiry, only 10 claimed a graduate program leading to a Ph.D. degree in mathematical statistics, and 14 an adequate training program at the advanced level for some field of applied statistics. Only 4 of the universities have special stipends for graduate work in mathematical statistics. The situation in applied statistics is hardly any more adequate.

The Committee emphasized the duplication of material in elementary statistics courses as they are now taught in various departments of a given college or university, as well as the heterogeneity of the quality of teaching. The opinion was expressed that the standardization and improvement of the teaching of statistics at the undergraduate level is a basic requirement for the solution to the problem of training statistical personnel. Specifically, it proposes that there should be developed a basic course in statistics at the freshman level for students who will go into the natural and social sciences, standardized to the same extent as introductory courses in mathematics, physics, and chemistry.

According to the Committee, one of the most puzzling problems regarding statistics is how it should be organized within a university. Two plans being tried out at certain universities were discussed: (1) the statistical laboratory and research center which would serve as an informal campus statistical center, and (2) the department of statistics. Plan (1) is necessarily rather informal and depends for its success on the voluntary cooperation of personnel from

various departments who are interested in research and teaching of statistics. Plan (2) would be more formal and desirable, but its success would depend on joint membership of its personnel with other departments. This is particularly important for the effective teaching of applied statistics, which should be carried out in conjunction with departments interested in applications of statistical methods.

The Committee summarized its conclusions as follows:

(1) There should be developed a basic introductory course in statistics at, preferably, the freshman level for colleges and universities throughout the country.

(2) The laboratory work in the average course in statistics is inadequate, particularly at the elementary level; experimental work should replace much of the computation at this level.

(3) The minimum requirement in effective organization is a central statistical laboratory with which all of those teaching or doing research in statistics would be associated, even though informally in some cases.

(4) More success is to be expected from a department of statistics associated with a statistical laboratory, and having some members in common with other departments.

(5) The number of institutions needed for giving first-class training through the graduate level are: (a) 5–10 in mathematical statistics, (b) 25–30 in varying fields of applied statistics.

(6) An institution giving complete training in either mathematical or applied statistics should give some training in the other.

(7) Institutional stipends for graduate students specializing in mathematical and applied statistics are inadequate.

(8) In strengthening its statistical work at the advanced and research levels, any given university should consider which field it can develop most effectively, so as to avoid duplication and inefficiency from a national point of view.

(9) The immediate critical shortage of highly qualified teachers can be eased only by suitable training of high-grade personnel now in fields of application, or mathematics.

(10) An adequate number of postdoctoral fellowships in statistics is needed.

(11) Arrangements should be established whereby postgraduate students, research workers, and teachers on leave would be able to obtain work experience in certain government agencies, industrial laboratories, and business research organizations.

(12) To help offset the present critical shortage of qualified personnel in applied statistics, it would be desirable to promote conferences at advanced levels and short courses at the elementary level in various fields.

Recent Deaths

Ole A. Nelson, 55, Battelle Memorial Institute staff member in charge of research activities on metals and chemicals in agriculture, died September 17 following an extended illness.

José F. Nonidez, 55, professor of microscopic anatomy, University of Georgia Medical School, and formerly professor of anatomy, Cornell University Medical College, died in Augusta, Georgia, September 27, after a brief illness.

Frederic Lendall Bishop, 71, professor of physics, University of Pittsburgh, since 1909, died October 10 at his home in Fox Chapel Manor, Pennsylvania.

Ellsworth Huntington, 71, research associate in geography at Yale University until his retirement in 1945, died October 17 at his home in Hamden, Connecticut. Dr. Huntington had been a member of the Yale faculty since 1907.

The American Institute of Physics, 57 East 55th Street, New York City, plans to publish a new monthly journal in physics which will be designed to fill part of the gap now existing between technical journals and popular science magazines. **David A. Katcher,** Naval Ordnance Laboratory, Washington, D. C., has been appointed editor of the as yet unnamed magazine, which will make its appearance early in 1948.

The Loyal Order of Moose has appointed a National Advisory Council for Research in Gerontology for the Fraternity's city for the aged at Moosehaven, Florida, near Jacksonville. The members of the Council include: Allan G. Brodie, dean, University of Illinois College of Dentistry, Chicago; Anton J. Carlson, emeritus professor, Department of Physiology, University of Chicago; Louis J. Haas, director, Men's Therapeutic Occupations, New York Hospital, White Plains; George Lawton, consulting psychologist, New York City; S. L. Pressey, president, Division on Maturity and Old Age, American Psychological Association, Ohio State University; Martin L. Remert, director, The Mooseheart Laboratory for Child Research; and N. W. Shock, chief, Gerontology Section, Baltimore City Hospitals.

The well-known Mooseheart Laboratory for Child Research, in Illinois, established by the same organization 17 years ago, has provided facilities for research in human development which have been utilized widely, and it is hoped that a similar arrangement may be made with respect to the new development. Inquiries may be addressed to Dr. Reymert.

The South African Association for the Advancement of Science is now publishing and editing *South African Science*, a monthly bulletin devoted to affairs of the Association, preliminary announcements of new discoveries, short communications, book reviews, longer articles, and so on, which will appear in English or Afrikaans, depending on the language in which they are received. The bulletin, the first issue of which appeared in August, is free to members of the Association; others may subscribe at 15/- per year. Further information with respect to contributions and subscriptions may be obtained from The Editors, *South African Science*, P. O. Box 6894, Johannesburg.

Make Plans for—

American Institute of Electrical Engineers, Midwest General Meeting, November 3–7, Chicago, Illinois.

American Institute of Chemical Engineers, November 9–11, Detroit, Michigan.

National Committee for Mental Hygiene, November 12–13, Hotel Pennsylvania, New York.

American Society of Animal Production, November 28–29, Chicago.

American Association for the Advancement of Science, 114th Meeting, December 26–31, Chicago, Illinois.

TECHNICAL PAPERS

Elementary Effect of Arginase in the Weight Physiology of the Mouse

W. G. IRONS and O. B. WISWELL

Department of Anatomy, College of Dentistry, University of California, San Francisco

A series of experiments dealing with the effect of *arginine* and its enzyme, *arginase*, upon growth and repair in the mouse has been the subject of a preliminary report (*1*). It was noted that administration of arginase intraperitoneally resulted in a systemic effect observable in a corresponding weight curve. Further substantiation of this phenomena has been obtained and will be the subject of this report.

Two series of animals have been used. Results from the first, 20 white mice of the Hamilton strain, with 20 controls, were obtained in January 1946. These were normal, hardy white mice. Results from the second series, 20 C3H strain with the same number of controls, were obtained in March 1947. This strain of mice was bred to develop tumors. The procedure upon both groups was identical, with the exception that the arginase used on the first series was obtained from the Biochemistry Laboratories of the University of California, and that used on the second series was prepared in our own laboratories. The same unit dosage was used in both instances.

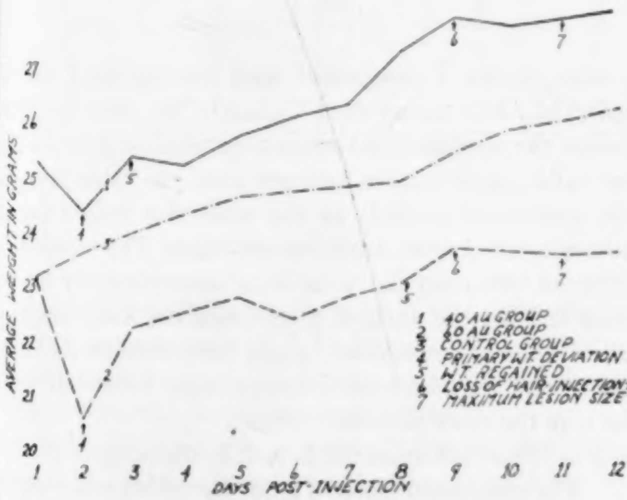

FIG. 1. Hamilton strain.

In the first and second series the animals were divided into groups of 10. One group received 40 AU (arginase units) (*2*) and a second group, 60 AU. Each animal was weighed prior to injection and at the same hour daily thereafter. (Minor exceptions are indicated in Figs. 1 and 2.) The injections were given intraperitoneally in the lower abdominal region.

Although a year elapsed between the two series, similar corresponding curves were obtained; and, in all animals injected, a lesion developed at the sight of injection upon the 8th day.

The weight charts indicate weight in grams and days post-injection of Series I and II, respectively. It will be observed that in the two groups of the first and the second series receiving 40 AU there was a weight deviation in the first 24-hour period of 0.9 gram and 0.8 gram, their injection-day weight being regained between the 3rd and 4th day. The two groups receiving 60 AU showed a weight deviation in the first 24-hour period of 2.6 grams and 2.0 grams, respectively. These did not regain their injection-day weight until late in the 7th or early in the 8th day. The lesion mentioned above developed in all of the injected groups on the 8th day. In the first series, both the 40 AU and the 60 AU groups manifested the lesion by a loss of hair around the injection site, followed in a progressive manner by a complete autolysis of the skin, abdominal musculature, and fascia to (but not including) the peritoneum, reaching this point upon the 11th day post-injection. In the second series, the 40 AU group showed a loss of hair, but further autolysis did not occur. The 60 AU group of this series, however, did develop the complete lesion as described.

The loss of hair on the 8th day is the first indication of any physiological reaction other than the weight change. The denuded surface shows no evidence of an inflammatory process. In the animals that experienced autolysis, the lesion maintained a sterile appearance during its entire progress.

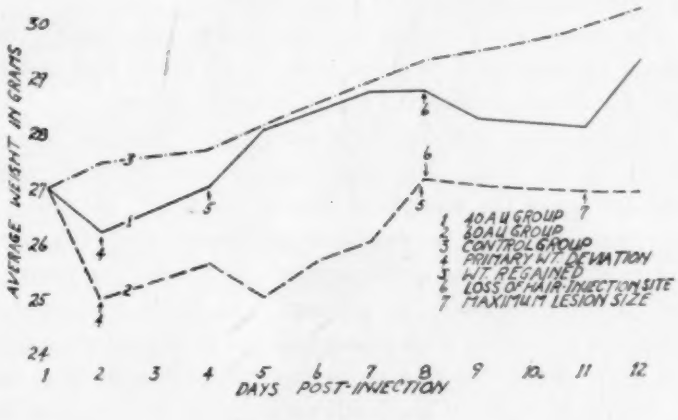

FIG. 2. C3H strain.

No rubor, suppuration, exudate, or swelling was in evidence. Regeneration appeared at the periphery, and the area covered over with a resultant permanent scar and loss of hair. The weight then tended toward normal.

The following conclusions may be drawn:

(1) The AU dosage is proportional to the time necessary for the animal to regain its injection-day weight.

(2) The differential in the primary weight loss of each of the two dosage-related groups indicates that there is a greater ratio of free arginine in the C3H tumor-producing strain.

(3) The greater arginine ratio in the C3H strain requires a slightly higher amount of arginase than the 40 AU used to produce the complete lesion.

(4) Re-establishment of the preinjection arginine-arginase balance in the intercellular fluid begins at the periphery of the lesion where the greatest dilution of the enzyme occurs. While

the injection was made intraperitoneally, the lesion results from an amount of the enzyme spreading about the trauma produced by the insertion and removal of the needle.

(5) The introduction of a suitable amount of arginase into the system buffers the arginine of the body, and, when into the tissues directly, inhibits the action of this substance in normal cell metabolism.

References

1. Irons, W. G. *J. dent. Res.*, 1946, 25, 497-499.
2. Mohamed, M. S., and Greenberg, D. M. *Arch. Biochem.*, 1945, 8, 349-364.

Antibiotic Compound Isolated From the Lichen *Ramalina reticulata*

A. Marshak, G. T. Barry,[1] and L. C. Craig

Tuberculosis Control Division, U. S. Public Health Service, and *The Rockefeller Institute for Medical Research, New York City*

Experiments conducted during the period July 1945–January 1947 showed that a crystalline substance isolated from *Ramalina reticulata* had *in vitro* antibiotic activity against a variety of gram-positive organisms and some acid-fast bacteria, including *Mycobacterium tuberculosis hominis*, but not against a number of different gram-negative organisms tested. *In vivo* tests with mice infected with pneumococcus gave negative results, while similar tests with tuberculous guinea pigs showed significant retardation of the progress of the disease (*6*).

Addition of base did not yield simple solutions of the lichen acid, and on the basis of this observation V. C. Barry suggested that the compound isolated might be usnic acid (*1*), shown to be a common constituent of many lichens.

The comparative studies described below were made with a sample of usnic acid received from V. C. Barry and isolated from *Cladonia sylvatica* [L] Harm. emend. Sandst.

The empirical formula (*5*) for usnic acid is given as $C_{18}H_{16}O_7$ [C: 62.79, H: 4.65]. The compound isolated from *Ramalina reticulata* gave the analytical figures [C: 62.88, H: 4.61], as previously reported, in excellent agreement with the above empirical formula. As doubt existed concerning the purity of the parent compound, an attempt was made to prepare a methoxyl derivative with diazomethane. It did not crystallize, but upon distillation in a molecular still gave analytical data [C: 65.75, H: 5.26, OCH_3: 9.50] which fitted the empirical formula $C_{16}H_{14}O_6$ (*6*). However, as the fractionally distilled methoxyl derivative was not crystalline, its homogeneity was also questionable.

A study was made of the homogeneity of the parent compound isolated from *Ramalina reticulata* by the method of countercurrent distribution (*4*). Sixty mg. of the material was distributed in a 24-tube countercurrent distribution machine, using a system containing 20 per cent cyclohexane and 80 per cent benzene as the upper layer and 10 per cent water and 90 per cent methanol as the lower layer, the two phases having first been equilibrated with each other before being used for the distribution. The volumes of the two phases were 12 cc. (upper phase) and 8 cc. (lower phase), giving an operational distribution ratio, K, of 0.41. In Fig. 1 is shown a 24-transfer distribution pattern of the substance. The concentration in each tube was determined by absorption at 284 mμ using the Beckman spectrophotometer. From the nearly complete correspondence with the theoretical curve it can be concluded that the material is apparently homogeneous, and therefore the empirical formula can safely be derived on the basis of the analysis of the original compound isolated rather than the doubtful methoxyl derivative.

The molecular weight of the compound as determined from the methoxyl content in the methoxyl derivative and by an alkali acetone titration of the parent compound was about 310.

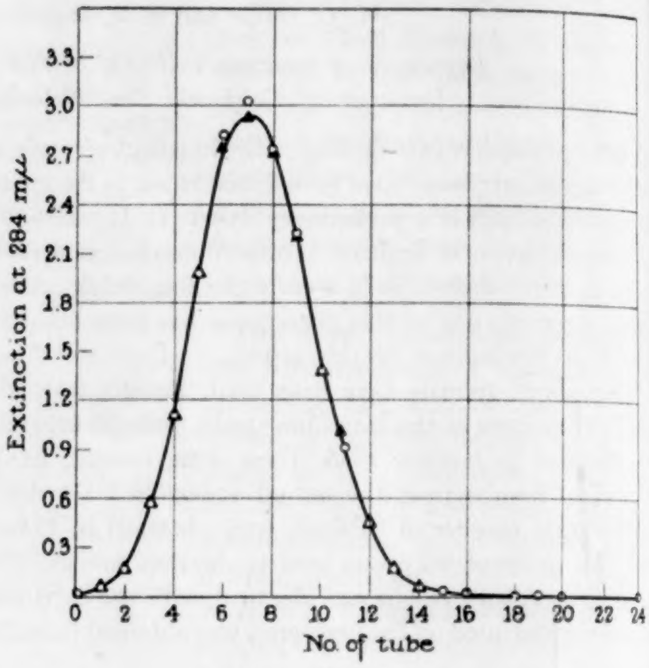

Fig. 1

This value favors a compound with an empirical formula $C_{16}H_{14}O_6$ (M., 302) rather than $C_{18}H_{16}O_7$ (M., 344) (*6*). However, since the acetone titration method employed can be considered valid only to within 5–10 per cent, the value 344 could still be considered possible as the molecular weight for the compound isolated from *Ramalina reticulata*. The coincidental similarity of the molecular weights as determined by the two methods first led the authors to consider the lower empirical formula. The later homogeneity study performed on the parent compound and the additional evidence given below now favors $C_{18}H_{16}O_7$ as the more probable value.

Usnic acid has been reported to melt in the range of 191–203° (*3, 7*). The compound isolated from *Ramalina reticulata* was reported to melt at 193–194° and showed a melting point similar to the compound received from V. C. Barry and isolated from *Cladonia sylvatica*. The melting point of the mixture of the two compounds showed no depression.

The ultraviolet absorption spectrum of the compound from *Ramalina reticulata* (Fig. 2) was similar to that of the compound isolated from *Cladonia sylvatica*. The absorption maxima are at 226–230 mμ and at 284 mμ.

The optical rotation for the compound from *Ramalina reticulata* in chloroform was $[\alpha]_D^{23} = +498°$, which corresponds to the value +495° reported for d-usnic acid (*4, 5*). The compound isolated from *Cladonia sylvatica* gave an $[\alpha]_D^{23} = -20.8°$ in chloroform and was considered to be the racemate of usnic acid with a small admixture of the l-form (*2*).

A study of the adsorption spectrum in the infrared region was made by Konrad Dobriner, of Memorial Hospital, New

[1] Fellow of the National Institute of Health and visiting investigator at the Rockefeller Institute for Medical Research.

York City, and at the American Cyanamid Company. Both substances showed absorption bands similar in frequency and shape at 40 points. The correspondence of these two compounds at so many frequencies would leave little doubt as to their identity.

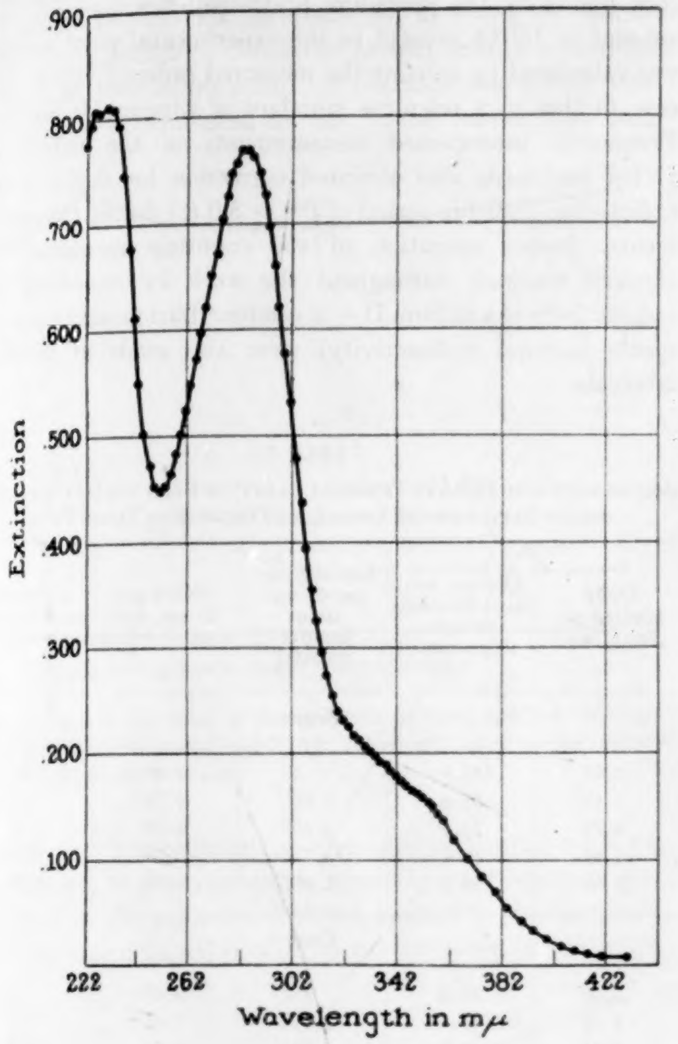

FIG. 2

Further investigations made by X-ray diffraction analysis of the two compounds by J. D. Bernal and I. Fankuchen indicated identical crystal structures. A more detailed account of their studies will be published elsewhere.

The results previously published (*6*) on *in vitro* inhibition of growth of various bacteria by the compound from *Ramalina reticulata* agree in general with those of Stoll and co-workers (*7*). Inhibition of gram-negative organisms is not obtained except at relatively high concentrations, while gram-positive organisms are inhibited by low concentrations. Human and bovine tubercle bacilli are inhibited by low concentrations, but the concentration required for inhibition of the avian strains of tubercle bacilli is higher.

References

1. BARRY, V. C. Personal communication.
2. BREADON, T. W., KEENE, J., and NOLAN, T. J. *Sci. Proc. roy. Dublin Soc.*, 1944, **23**, 197.
3. BRIEGER, R. *Flechtenstoffe* In KLEIN, G. *Handbuch der Pflanzeanalyse, Specielle Analyse*, 1932.
4. CRAIG, L. C. *J. biol. Chem.*, 1944, **155**, 519; CRAIG, L. C., GOLUMBIC, C., MIGHTON, H., and TITUS, E. *J. biol. Chem.*, 1945, **161**, 321.
5. CURD, F. H., and ROBERTSON, A. *J. chem. Soc.*, 1937, 894.
6. MARSHAK, A. *Publ. Hlth Rep.*, 1947, **62**, No. 1, 1–19.
7. STOLL, A., BRACK, A., and RENZ, J. *Experentia*, 1947, **3**, 115–117.

Relative Growth Rates of Bean and Oat Plants Containing Known Amounts of a Labeled Plant-Growth Regulator (2-Iodo^{121}-3-Nitrobenzoic Acid)

J. W. MITCHELL

Bureau of Plant Industry, Soils, and Agricultural Engineering

JOHN W. WOOD, W. C. WOLFE, and GEORGE W. IRVING, JR.

Bureau of Agricultural and Industrial Chemistry, Beltsville, Maryland

Succulent dicotyledonous plants are generally more sensitive to growth-regulating substances than are most plants of the monocotyledonous type (*2*), yet very little is known regarding the factors responsible for this difference in sensitivity. In recent experiments with the growth-regulating substance 2-iodo^{131}-3-nitrobenzoic acid (INBA) labeled with radioiodine, bean plants were found to absorb and translocate this compound more readily than barley plants (*3*), and a new tool was provided for observing still other differences in the way some dicotyledonous and monocotyledonous plants respond to growth regulators of this type.[1]

The present experiments were undertaken to determine, in part, whether the difference in sensitivity of plants, such as bean and oat, to such a growth regulator as INBA can be accounted for on the basis of a quantitative difference in their ability to absorb and translocate the compound. By applying radioactive INBA in varying amounts to the older leaves of bean and oat plants and then measuring the radioactivity of the young leaves that developed subsequently, it has been possible to compare the rate of growth of the two plant types when the young leaves of each contain equal concentrations of the growth regulator. Additional information which bears directly on this problem has also been obtained by studying the translocation of INBA in a third monocotyledonous plant, namely, corn seedlings.

The compound 2-iodo^{131}-3-nitrobenzoic acid was synthesized by diazotizing 3-nitroanthranilic acid and treating the reaction product with radioiodine 131.[2] A water dispersion of the compound was prepared by first dissolving the required amount in a small quantity of a commercial detergent (Tween-20) and then adding sufficient water to make a 4 per cent solution of the detergent in the final mixture. Measured amounts of the dispersion thus obtained were added to measured amounts of a 4 per cent solution of detergent in distilled water in order to make a series of clear, aqueous dispersions containing, respectively, 3.13, 6.25, 12.50, and 25.00 μg. of INBA/0.01 ml.

Bean and oat seedlings were grown from seed in potted soil under greenhouse conditions. Bean seedlings selected for uni-

[1] In the paper referred to it was tentatively concluded on the basis of the indirect evidence then available that INBA was absorbed and translocated as such in the plant. Since this work was published, it has been proved conclusively by the isolation of pure INBA from the stem and bud tissue of bean plants treated with this compound, that INBA is absorbed, translocated, and accumulated, at least in the bean plant, in the form of the intact molecule. While these experiments will be reported elsewhere, this fact is mentioned here to obviate the necessity for considering that a possible degradation product of INBA might be responsible for the results reported in this paper.

[2] Radioiodine was obtained through the Isotopes Branch, Manhattan District, Oak Ridge, Tennessee.

formity were treated when the second internodes were not more than 1 mm. long but beginning to elongate. Immediately before treatment a thin layer of lanolin was smeared on the upper surface of one primary leaf of each plant in order to cover an area of about 1 cm.² above the juncture of the petiole and leaf blade. This was done to block external movement of INBA from the treated leaves to other parts of the plant.

The oat seedlings were treated when they had developed one fully expanded leaf and a second leaf which was still partly enclosed in the leaf sheath. The third and fourth leaves were not outwardly apparent. Before treatment, lanolin was smeared across the upper and lower surfaces of the first leaf of each plant so as to cover a region extending approximately 1 cm. from the upper end of the sheath and toward the tip of the leaf.

Both the bean seedlings and the oat seedlings were divided into 5 groups of 30 plants each. The 30 bean and 30 oat seedlings in the first group of plants were used as controls, and each lanolin-blocked leaf was treated with 0.01 ml. of a 4 per cent solution of the detergent (Tween-20) in water. The solution was applied in each case to the upper surface of the leaf along the midrib in order to cover an area approximately 5 mm. wide and 10 mm. long in about the center of the leaf. The 30 bean and 30 oat seedlings in the other four groups were treated similarly by applying 0.01 ml. of a 4 per cent detergent-water dispersion containing, respectively, 3.13, 6.25, 12.50, and 25.00 μg. of radioactive INBA to each lanolin-blocked leaf.

For the study of the translocation of INBA in corn, the plants were grown from seeds in potted soil under greenhouse conditions. Fifty-one were selected for uniformity and treated when the first leaf was well expanded and a second leaf was partially expanded. The basal portion of the first leaf of each plant was blocked with lanolin by the technique described above on both the upper and lower surfaces and 0.01 ml. of a 4 per cent detergent-water dispersion containing 25.00 μg. of radioactive INBA was applied to the upper surface of each lanolin-blocked leaf.

Eight days later the treated leaves of the bean, oat, and corn plants were removed. Stems of the bean plants were severed at the second node, and all parts above the second node were collected. Each part was weighed individually, dried at 80° C. in a well-ventilated oven, reweighed individually, and then the parts were combined by groups and ground to 40-mesh for radioactivity measurements. The oat plants were severed at the uppermost end of the first leaf sheath, and all leaves above this point were weighed and processed as described for the bean plants. The corn plants were severed at the upper end of the first sheath and parts above this level were divided so as to obtain combined samples made up of the second, third, and fourth leaves, respectively. The corn leaf samples were processed as described for the bean plants.

Radioactivity measurements were made on 50-mg. aliquots of the dried, ground plant samples under rigidly standardized conditions using a Geiger-Müller scaling unit and a lead-shielded, Geiger-Müller counter tube having a thin mica window.[3] For measurement, the samples were placed in cylindrical stainless-steel cups having an internal diameter of 32 mm. and a uniform depth of 3 mm. Radioactivity measurements were also made under the same conditions on 50-mg. aliquots of each of a series of reference standards prepared by adding various known amounts of radioactive INBA to samples of tissue from corresponding parts of untreated bean, oat, and corn plants by the procedure previously described (3). The amount of INBA present in the experimental plant samples was calculated by relating the measured radioactivity in each case to that of a reference standard of comparable activity. Frequently interspersed measurements of the radioactive INBA standards also obviated correction for decay of the radioiodine (half-life period of I^{131} = 8.0 d.) during the experiments. Proper operation of the counting apparatus was checked routinely throughout the work by measuring the radioactivity of a radium D + E sample.[4] Background measurements (natural radioactivity) were also made at frequent intervals.

TABLE 1

ACCUMULATION OF INBA IN TERMINAL PARTS* OF BEAN AND OAT SEEDLINGS AND ITS EFFECT ON THE SUBSEQUENT GROWTH OF THESE PARTS

INBA applied per plant (μg.)	Dry wt. per plant terminal growth avg. (mg.)	Net activity per 50 mg. tissue (counts/minute)	INBA per 50 mg. dry tissue† (mg.)	INBA per plant terminal growth (μg.)
		Beans		
0.00	185.9	10	0.00	0.00
3.13	83.6	3,810	0.76	1.26
6.25	76.1	7,670	1.52	2.31
12.50	59.4	13,724	2.72	3.23
25.00	58.5	21,852	4.38	5.07
		Oats		
0.00	38.2	152	0.00	0.00
3.13	41.3	2,894	0.56	0.47
6.25	38.9	4,826	0.94	0.73
12.50	39.0	7,194	1.40	1.10
25.00	40.9	9,764	1.91	1.56

* All parts above sheath of first leaf of oat and above second node of bean plants are designated as terminal growth. This portion developed during the period of treatment (8 days).

† Based on standards made from dried leaves of bean and oat plants possessing average net activities of 5,047 and 5,123 counts/minute/γ of INBA, respectively.

The concentration of INBA in the most rapidly developing parts of both bean and oat seedlings increased progressively as the amount of INBA applied to the plants was increased (Table 1). In the case of the bean plant the accumulation of the compound in the terminal buds, even at the lowest level of applied INBA, resulted in a marked inhibition in the rate of growth of this tissue (45 per cent of control). At higher levels of application even greater inhibition in growth occurred, with maximum inhibition (approximately 32 per cent of control) attained upon the application of 12 or more μg. of INBA/plant (Fig. 1). In the case of the oat plant, however, no inhibition in

[3] The apparatus used had the following characteristics: counting circuit scale having 64 units, with a register circuit for operation of an external mechanical register; self-quenching, end-window-type counter tube having a threshold voltage of 1,200 and a mica window 28 mm. in diameter, with a thickness of 3.2 mg./cm.².

[4] The radium D + E sample used as a standard in these investigations was kindly furnished by L. F. Curtiss, National Bureau of Standards. The sample consisted of radium D plated on thin palladium foil. The absolute beta-ray activity of the sample, due essentially to radium E in equilibrium with radium D, was calculated by the Bureau of Standards to have a value of 147 microrutherfords (1 microrutherford = 1 disintegration/second, I).

e rate of growth of young leaves occurred, regardless of the ount of INBA applied to, and absorbed by, the plant.

Oat and corn plants absorbed and accumulated appreciably s of the applied INBA than did bean plants. For example, 8 ys after the application of 25 μg. of INBA to bean and oat nts, the concentration of the compound per unit of dried oat nt tissue was only half that found in the young leaves and ms of bean seedlings. Similarly, in corn plants, where no ibition in growth occurred, considerably less INBA was sorbed and accumulated in the young leaves than was

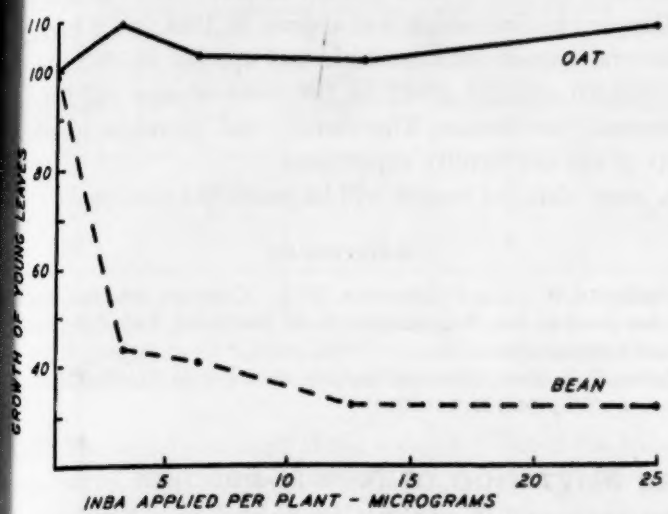

FIG. 1. Growth response of young leaves of bean and oat seedlings eated with different amounts of INBA. Values calculated on the basis of ntrols equal to 100 per cent.

bsorbed and accumulated in bean plants treated with an qual amount of the compound. Nevertheless, because of the ide range in the amounts of INBA applied to the bean and at plants, experimental conditions were attained in which the

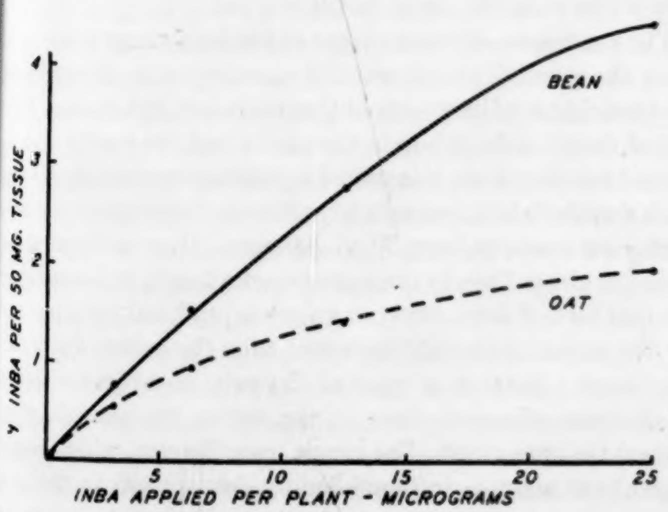

FIG. 2. Effect of applying increasing amounts of INBA to bean and oat eedlings on the concentration of the compound in the young leaves of each lant. (These curves yield essentially straight lines when plotted with ogarithm coordinates.)

mounts present in the rapidly growing parts of the two plant ypes were identical, making it possible to compare the growth-inhibiting effects of this compound in these plants under strictly comparable conditions. This fact is apparent when the NBA accumulation curves plotted in Fig. 2 are considered ogether with the growth curves given in Fig. 1. Equal concentrations of INBA (1.9 μg./50 mg. of dried tissue) were present in the young leaves and stems of bean plants and in the young leaves of oat plants when the plants were treated with approximately 8.3 and 25 μg. of INBA, respectively. The growth of the young bean leaves and stems containing 1.9 μg. of INBA/50 mg. of dried tissue was reduced to 37 per cent of that of controls, whereas the growth of young oat leaves that contained an equal concentration of INBA was not at all affected.

It can be concluded from these results that the difference in the sensitivity of bean and oat plants to INBA cannot be accounted for on the basis of differences in the ability of the plants to absorb and translocate the compound or on the basis of a difference in the extent to which INBA accumulates in the rapidly growing parts of the plants, since with equal concentrations in the young leaves of each type of plant the growth of bean was greatly reduced but that of oat was not affected. These results lend additional support to one of the two alternative conclusions previously reached (*3*), namely, that the growth-inhibiting effects of INBA in the bean plant and its failure to produce significant inhibition in barley, oat, and corn plants must be due to differences in the manner in which INBA reacts with the plant constituents in each case.

TABLE 2

ACCUMULATION OF INBA IN LEAVES OF CORN SEEDLINGS 8 DAYS FOLLOWING APPLICATION OF 25 μG. OF THE COMPOUND TO THE FIRST LEAF

Leaf in order of development	Dry weight per plant avg. (mg.)	Net activity per 50 mg. dry leaf tissue (counts/minute)	INBA per 50 mg. dry leaf tissue* (μg.)	INBA per leaf (μg.)
Second	49.6	5,298	1.27	1.26
Third	63.6	4,815	1.04	1.32
Fourth	31.8	3,621	0.78	0.50

* Based on standards prepared from corn leaves having an average net activity of 4,652 counts/minute/γ of INBA.

Three other implications of the experimental results are noteworthy: (a) Growth of the terminal buds of bean plants was reduced roughly in proportion to the amount of INBA accumulated in them until a concentration of approximately 2.7 μg./50 mg. of dry tissue was reached; at higher concentrations of INBA no greater inhibition in growth occurred. (b) INBA continued to accumulate in terminal buds of bean plants even after maximum inhibition had been attained. (c) In the case of the translocation experiments with corn seedlings, the concentration of INBA was greatest in the oldest leaf assayed and least in the youngest one in which the INBA content was determined. On the basis of these observations and other evidence to be presented at a later time, it is considered probable that INBA, and perhaps other plant-growth regulators of this type, not only accumulates primarily in the plant tissue that is developing most rapidly at the time the compound is applied, but also combines stoichiometrically and, for the most part, irreversibly with certain essential metabolites of these tissues to exert its growth-inhibiting effects.

References

1. CONDON, E. U., and CURTISS, L. F. *Science*, 1946, **103**, 712.
2. KRAUS, E. J., and MITCHELL, J. W. *Bot. Gaz.*, 1947, **108**, 301–350.
3. WOOD, J. W., MITCHELL, J. W., and IRVING, G. W., JR. *Science*, 1947, **105**, 337–339.

A Nutritional Disease of Oats Apparently Due to the Lack of Copper

Henry C. Harris

Department of Agronomy,
Florida Agricultural Experiment Station, Gainesville

A deficiency of available copper in the soil is reported to have caused a nutritional disease of oats in Europe (*1*) and in Australia (*2*), but apparently this disease has not been reported in this country. However, a similar disease has appeared in oats planted on the Experiment Station Farm at Gainesville, Florida, and this report is concerned with the symptoms of the trouble and the remedy for it.

The oat variety, Florida 167, when grown on certain parts of the Experiment Station Farm, has exhibited for several years an apparent nutritional disease. Others varieties have been affected, although they have been grown to a lesser extent. The symptoms of this disease develop on this variety as follows: After being seeded at the usual planting time in November, the oats come up and at first appear normal. The only difference detected after about two months is that the affected oats are smaller than nonaffected plants. In later stages of growth, about three degrees of severity of the symptoms have been noted, namely, severe, moderate, and slight. In severe cases the leaves begin to show a characteristic marginal chlorosis early in February. As the disturbance progresses, the margin and tips of the leaves become brown or may look as if they have been scorched. Tillers begin developing about the middle of February. The emerging tips forming the bud of the tillers frequently are rolled up tightly, and the rolled-up part becomes light colored, then brown, and eventually may die. After that the entire plant may die or may struggle along, putting out new tillers which in turn develop similar characteristics. Such plants produce practically no heads and little foliage. If the condition is moderate, the plants will have some of the characteristics described above. New tillers develop late, which results in the plant material being immature at normal harvesting time. The oats produce heads, but instead of ripening to a normal yellow color, these heads tend to have a whitish-green color, and very little grain is produced. Slightly affected plants appear normal except for considerable blasting and light grain. Sometimes the upper leaves, which cover the head just as it emerges, are chlorotic and may even appear scorched. Symptoms of this nature on this variety have been observed on several farms in central Florida.

Affected plants seem to develop wiry roots that show root rot in various stages, but this is probably secondary in nature. There was no apparent difference in the amount of *Helminthosporium* leaf spot on affected and normal plants.

Field experiments, in which the Florida 167 variety was grown, were conducted in a badly affected area[1] in an effort to determine the cause of the abnormality. In one set of experiments a uniform liberal application of the major fertilizer elements, nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur, was applied. In addition to the major elements, copper, zinc, manganese, boron, and molybdenum were applied to the soil in all possible combinations before seeding the oats. The copper was applied as copper chloride at the rate of 10 pounds/acre. The seed were treated with New Improved Ceresan. In another experiment an assortment of treatments were tried including fertilizer, rate of top dressing with nitrate of soda, seed treatment with New Improved Ceresan, and the minor elements in combination with these.

Oats which did not have copper developed severe symptoms of the disease and produced practically no grain. The foliage was small, and very few plants even produced heads. Oats grown on every copper treatment were either free or practically free of the described disease and produced relatively good yields. The only treatment of value was copper. Top dressing with extra nitrate of soda seemed to accentuate the trouble.

Copper chloride which was applied in **1944** to old fertility plots and copper sulfate which was applied in 1942 had a pronounced residual effect on the yield of oats and largely prevented the disease. The disease was prevalent in other parts of the old fertility experiment.

A more detailed report will be published elsewhere.

[1] The soil type is Arredondo loamy fine sand.

References

1. Melchers, W. J., and Gerritsen, H. J. *Koper als onmisbaar element voor plant en dier.* Wageningen: N. V. Drukkerij, Vada-Gebr. Zomer en Keuning, 1944.
2. Piper, C. S. *Aust. Coun. sci. industr. Res. Pamph. 78*, 1938, 24–28; *J. agric. Sci.*, 1942, **32**, 143–178.

The Migration of Newly-hatched Loggerhead Turtles Toward the Sea[1]

Robert S. Daniel and Karl U. Smith

University of Missouri and *University of Wisconsin*

The initial adaptations of newly-hatched loggerhead turtles may be divided into three major behavioral sequences: (1) escape from the deep nest on the ocean beach; (2) direct migration to the sea immediately after escape; (3) orientation toward deep water once the ocean surf is reached.

The conditions of development of the loggerhead turtle preclude the possibilities of prenatal stimulation in determining the remarkable adjustments of the newly-hatched animal. The nest of these turtles, a hole in the sand about one foot in diameter and two feet deep, is installed in midsummer months by the adult female, which immediately afterward returns to the sea. Each nest contains from 50 to 200 eggs. After an incubation period of about 7 weeks the young turtles hatch, mill around in the nest for 3–5 days, emerge as a group, and make a rapid run for the ocean. Although the crawl from the nest to the water may cover a distance as great as 25 yards, no errors are shown in direction of crawl. Once in the water, the animals swim toward the open ocean. The escape from the nest, which occurs typically at night, is accomplished by slow activity in the nest, which raises the nest floor gradually to the beach surface by knocking sand from above.

The guiding physical stimuli which determine the nest-to-sea movements of the young loggerhead have been the subject of some previous investigations. Hooker (*2*) and Parker (*3*) have referred to positive geotropic reactions, visual hue discrimination and preference, and discrimination of visual "openness" (the uniformity of the ocean horizon) as possible means of explaining the oriented crawling.

In order to determine the critical stimuli for the directing of

[1] Presented before the Midwestern Psychological Association and Section I, AAAS, St. Louis, March 1946.

the migration in the young turtle, observations were made under natural conditions and in a privately arranged laboratory on the east coast of Florida.

The major results of these studies show that the newly-hatched loggerhead is activated and guided in its movements from the nest to the sea by photokinesis and phototropism, the critical stimulus for which is normally the light reflected from the ocean surf. The following observations will serve to indicate the validity of this conclusion.

TABLE 1

NUMBER OF TURTLES RESPONDING POSITIVELY TO SIMULTANEOUSLY PRESENTED LIGHT AND GRAVITATIONAL STIMULATION

Angle of incline	No. of animals tested	No. showing positive geotropic response	No. showing positive phototropic response	No. showing ambiguous response*
6°	16	1	8	7
10°	6	1	3	2
13°	11	2	8	1
Total.......	33	4	19	10

* This group includes inactive animals as well as 4 animals which moved in no particular direction around the release point on the inclined plane.

Neither sound nor smell of the water will direct the crawling movements when vision of the surf is prevented. Sixty newly-hatched animals placed in an open pit on the beach milled around but showed no oriented movements in the direction of the water. When vision of the surf was permitted by placing them on the open beach, they entered the water immediately. Four animals, released on the beach on a quiet, moonless night when the surf was absent, failed to find the water, but when released later, at the same point on the beach, after the moon had risen, they crawled quickly into the ocean.

A group of 5 animals placed on the beach at night, were observed to follow a beam from a flashlight as it was moved around on the beach surface. Confusion of orientation was produced in a group of 50 turtles by releasing them on the beach on a moonlit night and by stimulating them simultaneously by a spot of light directed near them on the sand. About half of the animals followed the spot of light as it was moved about, and the other half crawled to the sea. Some individuals could be induced to reverse their direction of crawl, even after getting into the water, by moving the light from the edge of the ocean up onto the slope of the beach.

In the laboratory, the newly-hatched animals became very active when stimulated by ambient light, even of moderate intensity. Focal light stimuli in addition prompted the animals to approach and attack the light source. Turtles also showed an acceleration in approach time negatively related to distance from a focal stimulus. Circular crawling in the direction of the seeing eye was induced by temporarily blinding one eye and illuminating from above.

An approximate threshold of discrimination of light intensity was determined by observing the movements toward two light panels differing in brightness. They discriminated light ratios as low as 1:2 at an illumination level of 0.1 millilambert. This discrimination is smaller than the light differences typically occurring either at night or during the day between the surf and surrounding areas of beach and ocean.

Gravity as a stimulus was compared with light directly by releasing the animals on an inclined plane with a lighted window at the top. The results (Table 1) indicate that geotropism is relatively ineffectual. In a similar experiment, pairing light with sea water, the light approach again was dominant.

Further experiments were conducted to discover whether the turtles would crawl toward a "broken" visual pattern in preference to a "uniform" pattern, when opposed by a brightness difference favoring the broken pattern. Every one of a group observed approached the brighter but interrupted stimulus pattern in preference to the dimmer uniform field. This result pointed to an interpretation in favor of light rather than pattern as critical. As applied to the natural environment, this indicates that it is unlikely that the presence or absence of trees, shrubs, or sand dunes defines the seaward movements.

Inasmuch as the migratory nest-sea reactions normally occur at night under levels of illumination precluding the possibility of hue discrimination for the rod-cone eye, as is found in turtles, it was considered that hue discrimination could not determine the movement.

The previously reported embryonic study of development in the loggerhead (*4*) disclosed that the crawling and swimming movements evolve as specialized reactions within a matrix of more generalized body movements, and that these reactions, along with the migratory response pattern itself, may be elicited in animals prematurely released from the egg. The fundamental character of the oriented reactions was further established by the fact that the movements toward light stimuli are relatively unmodifiable through learning of a detour path in a Y-maze. The conclusion was clear from these observations that the light-induced movements were sufficient to account for the stereotyped character of the actual sea-approach behavior. Positive phototropism, conceived as a stage in maturation, is further suggested by the fact that the response becomes weak at about 6 days of age and a negative response to very bright light appears at about 3 weeks of age.

It may be concluded that the phototropic reactions to differences in light intensity and the kinetic effects of light, which may be observed under both laboratory and natural conditions, have significant relations with the environmental circumstances under which the nest-sea migration occurs. At night the white and sometimes phosphorescent surf constitutes the most intense light stimulus in the nest locale. This light is apparently the critical stimulus for both activating and guiding the ocean crawl. At dawn or dusk or, in fact, throughout the day, the brilliant surf also acts as a directing and motivating influence for the animals to reach the ocean. The observations showed that the positive responses to light are dominant and invariable during the first few days after hatching. In addition, it was observed that the female adult carefully selects nestsites which provide clear vision of the surf, and that other geographic features are not involved in the nest location.

The physical stimuli effective in leading the animal from the surf to the deeper areas of the ocean and the processes underlying these movements were not clearly defined.

These studies will be reported in full elsewhere (*1*).

References

1. DANIEL, R. S., and SMITH, K. U. *J. comp. physiol. Psychol.*, in press.
2. HOOKER, D. *Yearb. Carnegie Inst.*, 1907, #6, 111; 1908, #7, 124; *Science*, 1908, 27, 490; *Pap. Tortugas Lab., Carnegie Inst.*, 1911, 3, 69.
3. PARKER, G. H. *J. exp. Zool.*, 1922, **36**, 323.
4. SMITH, K. U., and DANIEL, R. S. *Science*, 1946, **104**, 154.

IN THE LABORATORY

Routine Analysis of the Alpha Activity of Protactinium Samples

M. Blau and H. Sinason

Gibbs Manufacturing and Research Corporation and *Canadian Radium & Uranium Corporation, New York City*

The classical method of measuring the alpha activity of protactinium consists of the following procedure: The samples containing protactinium are painted on metal discs as uniformily as possible and in extremely thin layers in order to provide minimum absorption for the alpha particles of protactinium. The current produced by the alpha particles of the sample is measured by an electrometer or electroscope and compared with that of a uranium standard.

The current obtained by the protactinium sample, expressed in e. s. u., is used for the determination of the number of alpha particles emitted by the sample:

$$J_{e.s.u.} = N \cdot e \cdot n, \qquad (1)$$

where N is the number of alpha particles emitted; e, the electric charge of an ion; and n, the number of ion pairs produced by an alpha particle of protactinium along its path through the ionization chamber.

Knowing the half-life of protactinium, the number of alpha particles emitted by, for instance, 1 mg. of protactinium can be calculated. One mg. of protactinium emits in total (whole sphere) 1.85×10^6 alpha particles/second.

The fraction $\frac{2N}{1.85 \times 10^6}$ gives the amount of protactinium (in mg.) which the sample contains, provided that the absorption of the layer of foreign material can be neglected. However, this assumption can actually never be realized completely. Therefore, the maximum value of $\frac{2N}{1.85 \times 10^6}$ corresponding to a zero absorption has to be determined by extrapolation, measuring samples of decreasing total weight. The ratio $\frac{\text{current}}{\text{weight of sample}}$ increases with decreasing layer thickness and, plotting these values versus the weight of the samples, we obtain a curve whose intersection with the ordinate gives us the actual value of the protactinium content per unit weight of sample material.

It is evident that this method is time consuming and subject to various errors, especially if the sample is very dilute with respect to the protactinium content. Moreover, this method presents a great many difficulties in plants where other radioactive products are present, especially alpha radiators of shorter half-life than protactinium, because even the slightest contamination introduced in the process of painting the discs can give rise to great errors. If, for instance, in a sample of 1 mg. of protactinium/gram, only 10^{-6} mg. of polonium is present, the error in the determination of the protactinium content would amount to more than 10 per cent.

In order to overcome these difficulties, we decided to apply for routine measurements the method of measuring the samples in thick (alpha-saturated) layers, especially since it affords less handling of the material, which, after suitable grinding, is filled into special, strictly uncontaminated dishes.

The theory of the ionization, above alpha-saturated layers of material, due to its emission of alpha particles was given by R. D. Evans (*1*). The number of ion pairs, Nip, produced by the alpha particles emitted by 1 cm.² of the sample layer is given by

$$\text{Nip} = \frac{J}{e \cdot f}, \qquad (2)$$

where J is the current due to the alpha particles of the sample; e, the charge of the ions; and f, the surface of the sample. As these samples contain much more material than the painted layers, a slight contamination does not affect the accuracy in such high degree and can be recognized more easily be refilling. The relationship between this number and the content of protactinium per gram of the sample Pa is given by the equation

$$\text{Nip} = \frac{L}{A_{Pa}} \cdot (EKR\lambda_{Pa}\text{Pad}\mu). \qquad (3)$$

In this equation L is the Avogadro number and A_{Pa} the atomic weight of protactinium; the radioactive constants, EK, R, λ, Pa which refer to the specific ionization and range of the alpha particles and to the disintegration constant of protactinium, have been tabulated (*1*). Therefore, Pa, the protactinium content per gram of sample, could be calculated if d, the density of the sample, and μ, the absorption coefficient for the alpha particles, *i.e.* the ratio between the alpha particle range in the solid to its range in air, are known.

The density of the sample can be obtained experimentally, but would require separate measurements; on the other hand, an experimental determination of μ presents quite great difficulties.

To overcome these difficulties and to establish simultaneously and once for all the value of the product "μd" for our special samples (ZrP_2O_7), we proceeded as follows:

(1) Very careful measurements were made using the first-described method of thin-layer samples in order to obtain directly the content of protactinium per gram of the sample. Thereafter, exactly the same sample of material was used in an alpha-saturated layer, and the product "μd" was calculated, substituting for Pa in Equation 3 the value found in the thin-layer experiments. The average value obtained in four series of measurements is: $\mu d = 2.36 \cdot 10^{-3}$.

(2) An accurately known quantity of polonium was added to ZrP_2O_7 samples of low protactinium content and later also

unactivated ZrP_2O_7 samples.[1] The polonium was (a) added form of a solution (HCl) and (b) precipitated on ferric ydroxide $[Fe(OH)_3]$.

In case (a) the exact quantity of polonium was determined y spinning a nickel foil in an aliquot part of the polonium lution used for the experiment and by measuring this foil y photoelectric or ionization methods. The solution was oroughly mixed with the zirconium sample and dried at only ightly elevated temperatures until the original weight was ached. Because of the high specific activity of polonium, no rrection factor with respect to density changes have to be ade. Two series of measurements gave the average value of $d = 2.20 \times 10^{-3}$.

In case (b) a nickel foil with a well-known amount of olonium was dissolved and the polonium precipitated on $e(OH)_3$. By this procedure about 5 per cent of the original olonium quantity is lost, and this factor, as well as the ange in density by adding approximately 10 mg. of $Fe(OH)_3$ o 1 gram of sample material, was taken into consideration. x series of measurements gave the average value of $\mu d =$ $\cdot 10^{-3}$; this value is smaller than that in (a) and 11 per cent aller than in case 1. In three series out of these six we measred only the quantity of added $Fe(OH)_3$ before the precipiation with polonium and did not observe the weight after ving dried the sample. It might be that, because the drying as not complete, the value of density appears too small. On e other hand, the values of series 1 may be too high, as the mples were very dilute with respect to protactinium, so that e active material is covered with an absorption layer.

The average value of the three sets of measurements, $d = 2.22 \cdot 10^{-3}$, was accepted and introduced in Equation 3, hich now allows us simply to calculate the content of proctinium precipitated on ZrP_2O_7, if the sample is measured alpha-saturated layers.

For routine measurements, the necessary calculations can be ill further simplified by comparing the ionization above the otactinium samples with that appearing over the uranium andard. If the ionization effect is the same in both cases, suming equal surfaces of the preparation, we find that the ntent of protactinium in the zirconium sample is 1.3×10^{-5} am/gram of sample. Therefore, the protactinium content in ams per gram of sample can be expressed by

$$Pa = \frac{J_{ZrP_2O_7+Pa}}{J_{U_3O_8}} \cdot 1.3 \cdot 10^{-5} \text{ gram.}$$

The above considerations, of course, are valid only if the dioactive element is always separated by the same chemical rocesses, a common occurrence in routine work of this nature.

The use of polonium for the determination of the factor μd n the case of protactinium samples is self-indicating because of e nearly equal range of the alpha particles emitted by these dioactive elements. However, the same procedure can be ecommended in the case of other alpha emitters of appreciable alf-life, e.g. plutonium and other transuranic elements, since he addition of polonium, due to its high specific activity, does ot alter the absorption in the sample.

Reference

. Evans, R. D. *Phys. Rev.*, 1934, 45, 29.

[1] We are grateful to W. R. Horn and M. Pavey, of International Rare etals Refinery, Inc., for the preparation of all of these samples.

A Simple Quantitative Method for Intravenous Injection of Small Volumes of Fluid

Henry L. Barnett and Francis X. Fellers[1]

New York Hospital and *Cornell University Medical College Department of Pediatrics*

The intravenous injection of small, accurately measured quantities of solutions is frequently required in physiological investigations. In most of the reported studies the technic has consisted of using a syringe calibrated to contain the solution and final rinsing of the syringe with blood several times to insure complete delivery (*1*). In the course of a study of the distribution of radioactive and nonradioactive substances injected intravenously into small infants, a simple technic was adopted which has several advantages.

The arrangement of the apparatus, which consists of two ordinary glass syringes with Luer locks and a metal three-way stopcock,[2] is shown in Fig. 1. One or more solutions are de-

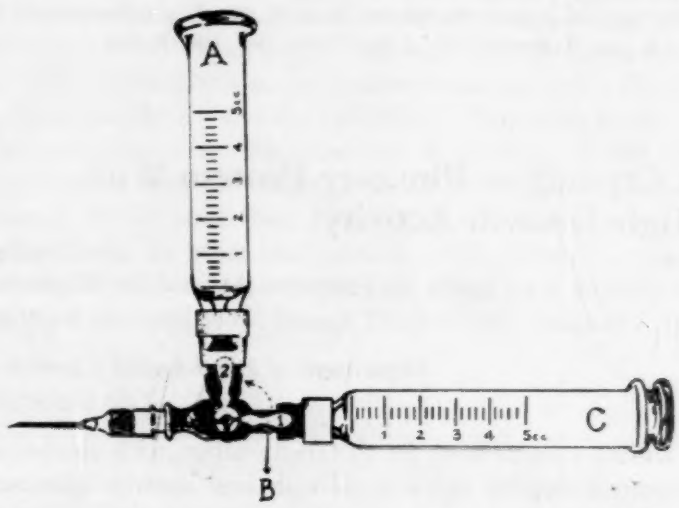

Fig. 1

livered from sterile volumetric pipettes of desired capacity into the upright syringe barrel, A, with the stopcock handle, B, in position 1. After the vein is entered, the handle is turned to position 2, and the plunger of the horizontal syringe, C, is withdrawn, bringing the solution into the barrel. Returning the handle to position 1 and advancing the plunger into the barrel delivers the solution through the needle into the vein. The upright barrel can then be filled with saline to the height of the original fluid level and washed through the apparatus as many times as desired by repeating the above maneuvers.

One-ml. aliquots of four different solutions (thiocyanate, creatinine, mannitol, and sodium-*p*-aminohippurate[3]) were pipetted into the upright barrel of the assembly and then delivered through the apparatus into 2,000-ml. flasks. With two 4-ml. rinsings of the assembly into the flasks, concentrations of the four substances were found to be identical with those in flasks into which similar aliquots were delivered

[1] With the technical assistance of Dorothy Weber.

[2] This is the type commonly used for intravenous injections in pediatrics and is available from Becton-Dickinson & Company, Rutherford, New Jersey.

[3] The mannitol and sodium-p-aminohippurate were kindly supplied to us by W. P. Boger, of Sharp & Dohme, Inc., Philadelphia.

directly from the pipettes. Quantitative recovery was confirmed by finding no residual substances in additional rinsings of the assembly.

The difficulty of intravenous injections in small infants is not increased by using the arrangement of the syringes described. The technic has the following advantages:

(1) The volume or volumes of solution to be injected can be measured more accurately from volumetric pipettes than from calibrated syringes, and the former are more readily available.

(2) Several solutions can be measured quantitatively and given in a single intravenous injection.

(3) Rinsing is done with saline rather than aspirated blood. The small amount of additional fluid required to rinse the assembly will not, in most instances, introduce a significant error in the determination of fluid "spaces."

The last two items are particularly applicable to studies of infants or small animals in which multiple injections and rinsing with blood are difficult.

References

1. Kaltreider, N. L., Meneely, G. R., Allen, J. R., and Bale, W. F. *J. exp. Med.*, 1941, **74**, 569–590; Pace, N., Kline, L., Schachman, H. K., and Harfenist, M. *J. biol. Chem.*, 1947, **168**, 459–469.

A Crystalline Pituitary Protein With High Growth Activity[1]

Jacob B. Fishman, Alfred E. Wilhelmi, and Jane A. Russell

Department of Physiological Chemistry, Yale University

During current work on the fractionation with alcohol of calcium hydroxide extracts of fresh beef anterior pituitary glands we have obtained crystalline fractions with very high growth hormone activity. The procedure is briefly as follows: Dissected anterior lobes are frozen in solid carbon dioxide and ground to a fine powder. After the carbon dioxide has evaporated, the gland powder is suspended in calcium hydroxide solution, pH 11.5, and stirred vigorously overnight. This and all subsequent operations are carried out in a cold room at 0–5° C. The pH of the extract is adjusted to 8.7 by bubbling in carbon dioxide gas, and after the mixture has stood again overnight it is centrifuged and the residue is discarded. To the supernatant solution, vigorously stirred, 1:1 alcohol-water is slowly added. Successive additions yield 5 fractions: A, at pH 8.6 and 13 per cent alcohol; B, pH 8.6, 19 per cent; C, pH 6.8, 23 per cent; D, pH 4.6, 23 per cent; and E, pH 4.6, 40 per cent. The fractions are separated by centrifugation, resuspended in water, and lyophilized. To prepare crystalline material, fraction A or B is dissolved in calcium hydroxide, pH 11.5, to make a 0.5 per cent solution, the pH is adjusted to 8.6 with carbon dioxide, the solution is centrifuged, and to the supernatant solution, vigorously stirred, enough 1:1 alcohol is very slowly added to make an alcohol concentration of 7 per cent. The crystals, which impart a beautiful silken sheen to the solutions from which they appear, seem on microscopic examination to be very thin, rectangular plates, many of them broken because of the shearing stresses of the vigorous stirring which is necessary during the addition of the alcohol. They are centrifuged off, suspended in water, and lyophilized. In the first experiment, starting with 304 grams of fresh glands, the yield of crystalline material from a portion of fraction B was 200 mg. In the second experiment, in which 350 grams of fresh glands were used, the yields were 74 mg. from fraction B and 950 mg. from fraction A.

The three crystalline fractions so far obtained have been assayed by the 10-day growth test on hypophysectomized rats. Their activities were compared with that of a purified growth hormone preparation made by us according to the method of Li, Evans, and Simpson (*1*). The results are summarized in Table 1.

TABLE 1

Fraction	No. of rats	Dose/day		Mean weights of rats (grams)		
		(mg.)	(mg. N)	Initial	Final	Change
30A	3	0.020	0.0032	194	212	+18
49A	3	0.100	0.0147	92	117	+25
	3	0.010	0.0015	95	108	+13
50A	3	0.100	0.0142	91	112	+21
	3	0.010	0.0014	87	98	+11
Li	2	0.099	0.0131	95	120	+25
	3	0.010	0.0013	91	108	+17

Autopsies carried out on the test animals showed that the weights of the thyroids, adrenals, testes, seminal vesicles, prostate, and liver were not different from the weights of these organs in uninjected controls. Histological studies on the thyroids are not yet complete. In the tests with fractions 49A, 50A, and the Li preparation the widths of the tibial epiphyseal cartilages were also measured. These were in μ: for 7 controls, 125; for 49A, 50A, and the Li preparation, at the 0.1-mg. dose level, 320, 309, and 379, respectively; and at the 0.01-mg. dose level, 291, 270, and 278, respectively. These results provide an additional measure of the activity of the crystalline preparations.

The three crystalline fractions have been examined electrophoretically in phosphate buffer (ionic strength, 0.2) at pH 8.0. Two components were noted in each instance. Additional electrophoretic studies on recrystallized material are in progress, experiments are under way to determine whether the two components can be separated, and further work is being done to determine the maximum yield of active material obtainable by the new method.

The results so far indicate that crystalline preparations comparing favorably in growth activity with the purified hormone can be prepared in excellent yields by a relatively simple alcohol fractionation of an alkaline extract of anterior pituitary glands. Although not all of the fractions obtained have been studied thoroughly, it is hoped that the procedure may lead to the isolation in pure form of other active principles of the anterior pituitary gland.

Reference

1. Li, C. H., Evans, H. M., and Simpson, M. E. *J. biol. Chem.*, 1945, **159**, 353.

[1] The work reported in this paper is being done under a grant from the American Cancer Society on the recommendation of the Committee on Growth, National Research Council.

Exchange of Carbon Dioxide Between Barium Carbonate and the Atmosphere[1]

W. D. Armstrong and Jack Schubert

Department of Physiological Chemistry and Laboratory of Dental Research, University of Minnesota

It was observed that precipitates of barium carbonate ($BaC^{14}O_3$ + $BaC^{12}O_3$) used as standards for radiocarbon (C^{14}) measurements appeared to lose a significant amount of radioactivity when stored for several weeks in contact with air in covered Petri dishes. In an experiment designed to test these observations and in which the Geiger-Müller counts were referred to a uranium standard a sample of radioactive barium carbonate lost 2.7 per cent of its original activity in 6 days. These findings could be explained as being the result of the incorporation in the precipitate of $C^{12}O_2$ from the atmosphere in exchange for $C^{14}O_2$. A mechanism for the exchange was postulated as follows:

$$C^{12}O_2 + H_2O \rightarrow H_2C^{12}O_3$$
$$BaC^{14}O_3 + H_2C^{12}O_3 \rightarrow BaC^{12}O_3 + H_2C^{14}O_3$$
$$H_2C^{14}O_3 \rightarrow H_2O + C^{14}O_2$$

Evidence that the exchange occurs and that the hypothesized mechanism is correct was obtained when it was demonstrated that (a) no loss of radioactivity by barium carbonate occurs in the absence of water vapor or carbon dioxide, and (b) radioactive carbon dioxide is released to the atmosphere, when the exchange occurs, in an amount equivalent to the decrease in radioactivity of the barium carbonate.

Precipitates of barium carbonate containing radiocarbon with the weights shown in Table 1 and surface areas of 5.73 cm.^{2} were collected in brass, Buchner-type funnels, as previously described (*1*). After the radioactivity of the precipitates had been measured with a thin mica window counter, each funnel and precipitate was separately placed in a vacuum desiccator of 2-l. capacity under the conditions indicated in Table 1. In Experiments 1 and 2 the bottom of the desiccator was covered with a layer of acidulated water in order to saturate the atmosphere of the container with water vapor. In Experiment 3 the air in contact with the barium carbonate was kept dry by a layer of phosphorus pentoxide covering the bottom of the desiccator. The gases ($C^{12}O_2$ or N_2) were admitted in sufficient amount to restore the pressure to atmospheric after the desiccator had been exhausted with a water pump. After the times indicated, the carbon dioxide present as a gas in the desiccator was collected in sodium hydroxide solution and the carbonate of the whole of this solution, or of an aliquot, precipitated as barium carbonate (*1*). The radioactivity of these precipitates and of the original barium carbonate, after drying and reweighing, were then determined. The activity of all samples was corrected for changes in counter performance by reference to counts of a uranium standard which was counted at least four times alternately with each sample.

The total activity of the precipitates, all of which were "thick" samples, were calculated by multiplying the observed count, corrected for counter performance and background, by the ratio of the thickness of the precipitate (expressed as mg./cm.^{2}) to 20. The relatively large statistical error shown for the radiocarbon found in the desiccator gas in Experiment 1 is due to the fact that the large amount of carbon dioxide necessitated counting a barium carbonate precipitate which contained only $\frac{1}{20}$ of the total carbon present in the gas phase of the desiccator.

In Experiment 1 the original precipitate lost 37.4 per cent of its radioactivity and, within the limits of experimental error, all of the radioactivity so lost was recovered as carbon dioxide in the atmosphere above the precipitate. The loss in weight of the precipitate after the treatment is probably within the experimental error of weighing the brass dishes and would account for no more than 1.74 per cent of the decrease in radioactivity. In other trials similar to Experiment 1 radioactivity equivalent to 12–48 per cent of the total was lost by the precipitates in 48–60 hours.[2] These results, obtained in the presence of water vapor and carbon dioxide, and the fact that no loss of activity occurs when barium carbonate is treated as indicated in Experiments 2 and 3, are evidence that the mechanism postulated above is correct. Under the conditions of these experiments it appears that the exchange, as in Experiment 1, occurs uniformly throughout the precipitate, since thorough mechanical mixing of the barium carbonate after

TABLE 1

Effect of Water and Carbon Dioxide on Loss of Radiocarbon by Barium Carbonate (t = 22° C.)

Exp. No.	Treatment: Conditions	Treatment: Time (hr.)	Original weight of precipitate (mg.)	Weight of precipitate after treatment (mg.)	Total activity of precipitate (counts/minute)*: Before treatment	Total activity of precipitate (counts/minute)*: After treatment	Total activity recovered in CO_2 in desiccator*
1	$H_2O + C^{12}O_2$	65	178.1	175.0	1,782 ± 19	1,115 ± 16	620 ± 90
2	$H_2O + N_2$	71	176.3	176.3	1,710 ± 18	1,693 ± 18	B.G.†
3	$P_2O_5 + C^{12}O_2$	41	157.5	157.0	1,448 ± 16	1,460 ± 16	B.G.

* The activity results are shown ± the square root of the sum of the squares of the errors of the sample and background.
† B.G. = Not significantly different from the background count.

[1] This work was carried out with the aid of grants from the John and Mary R. Markle Foundation and the Graduate School of the University of Minnesota.

[2] A sample of enriched C^{13} barium carbonate containing 2.86 atom per cent excess of C^{13} was subjected to similar treatment. The atom per cent excess of C^{13} was reduced to 2.64, showing that a definite exchange with atmospheric CO_2 had occurred. Since the $C^{13}O_2$ released was diluted 75-fold, it was not possible to ascertain with accuracy the resulting increase in C^{13}/C^{12} ratio in the atmosphere of the desiccator, although qualitatively this gas showed an enriched C^{13} content above its original composition. The enriched $BaCO_3$ and the analyses were kindly provided by Alfred O. Nier, Department of Physics, and Nathan Lifson, Department of Physiology, University of Minnesota.

the treatment caused no change in activity. This would not have been the case had the exchange occurred only on the surface layers of the precipitate.

These observations indicate the necessity for preserving carbonates which, in the solid state, are to be used as isotopic carbon reference materials in such manner that carbon dioxide or water, or both, are excluded from contact with the solid substance. Further, the observations confirm the possibility that one of the paths by which sunflower leaves absorb carbon dioxide from the air is by exchange of carbon dioxide with insoluble carbonates in the leaves (*2*).

References

1. ARMSTRONG, W. D., and SCHUBERT, J. *Anal. Chem.*, in press.
2. SMITH, J. H. C., and COWIE, D. B. *Plant Physiol.*, 1941, **16**, 257.

The Use of Isotopes to Determine the Rate of a Biochemical Reaction[1]

HERMAN BRANSON

Department of Physics,
Howard University, Washington, D. C.

Radioactive and stable isotopes are being used increasingly to determine the rates of biochemical reactions for which suitable techniques were previously not available. The theory of this application of isotopes has been discussed by Zilversmit and his collaborators (*2*) and by Branson (*1*). Both formulations show that in order to determine the rate for a substance, B, formed from A, the experimenter must have information on the time dependence of A and B.

Branson's integral equation formulation gave

$$M(t) = M(0)F(t) + \int_0^t R(\theta)F(t-\theta)\,d\theta,$$

where M(t) is the amount of substance present at time t; M(0) is the amount initially present; F(t) is the "metabolizing" function—the function which multiplies the original amount to give the amount present at time t; and R(t) is the rate of accumulation. The equations applicable to the system $A \rightarrow B$, where A is transformed into B by a first order chemical reaction, are:

$$A^*(t) = \int_0^t R(\theta)F(t-\theta)\,d\theta$$

$$B(0) = B(0)F_1(t) + \int_0^t R_1(\theta)F_1(t-\theta)\,d\theta \qquad (1)$$

$$B^*(t) = C\int_0^t A^*(\theta)F_1(t-\theta)\,d\theta,$$

which assume that the amount of B present is constant and R = CA. The starred quantities refer to the substances isotopically tagged. Hence, if we are interested in the R and F associated with B(t), we must determine experimentally A*(t), B*(t), and B(0).

The procedure is open to severe criticism in most complex biological systems, for it requires the unequivocal proof that B is the exclusive product of A. In general that will not be true; and, if it were, the scheme would be difficult to establish. Under the dynamic conditions existing in biological systems, we can expect side reactions and complex chains, some of which may lead eventually from A to B.

One procedure for eliminating the dependence upon measurements of A*(t) would be to introduce the tagged substance suspected as being the precursor of B*(t) in one system or series of animals and the tagged substance, $\overline{B}(t)$, in a similar system. From the first system we have

$$B^*(t) = \int_0^t R(\theta)F(t-\theta)\,d\theta \qquad (2)$$

and from the second,

$$\overline{B}(t) = \overline{B}(0)\;F(t), \qquad (3)$$

which are sufficient to determine F(t) and R(t) without dependence upon precursors of B.

Although of value, the preceding technique is probably as vulnerable as the first, since the measurements are made upon different systems. The experimenter would have greater confidence in his rate determinations if they were based solely upon measurements of the substance under study and limited to simultaneous measurements in a single system.

These desired conditions may be obtained by the use of a doubly-tagged[2] substance in a single system. We may introduce the suspected tagged precursor of B and follow experimentally the level of B*(t). At the same time, we may inject some $\overline{B}$—that is, a small amount of the chemically similar substance but tagged by the use of a different radioactive isotope or by a rare stable isotope. Thus, Equations 2 and 3 permit the simultaneous determination of R(t) and F(t) in a single system by measurements of B alone.

The number of possible combinations of isotopes available depends in large measure upon the problem and the skill of the experimenter. Under all circumstances, radioactive and stable pairs such as (H^2, H^3), (C^{13}, C^{14}), (S^{34}, S^{35}) and the radioactive pair (Fe^{55}, Fe^{59}) can be used for these elements. No appropriate radioactive member exists for oxygen or nitrogen; however, in many experiments they can be coupled with hydrogen, carbon, or sulfur. The assays will require the simultaneous use of the mass spectrometer and the Geiger-Müller counter, but such equipment is now rather widely available.

Experiments of this type are being planned for our laboratory using a small 60° Nier Type spectrometer and conventional Geiger-Müller equipment.

References

1. BRANSON, HERMAN. *Bull. Math. Biophys.*, 1946, **8**, 159–165; 1947, **9**, 93–98.
2. ZILVERSMIT, D. B., ENTEMAN, C., and FISHLER, M. C. *J. gen. Physiol.*, 1943, **26**, 325–331; ZILVERSMIT, D. B., ENTEMAN, C., FISHLER, M. C., and CHAIKOFF, I. L. *J. gen. Physiol.*, 1943, **26**, 333–340.

[1] This work is being supported in part by a grant from the Research Corporation of New York.

[2] "Doubly-tagged" designates a substance, part of whose molecules are tagged by one isotope and part by another; e.g. methionine, with some molecules having S^{34} and some S^{35}, or betaine, with some molecules having N^{15} and some C^{13}.